Volume 76 Number 12

CURRE

Lipoprotein(a) in Atherosclerotic Vascular Diseases

Sandal Spike Phytoplasma

Antarctic Bacteria

Current Science Association

DEPARTMENT OF SCIENCE AND TECHNOLOGY

(INTERNATIONAL DIVISION)

India–China S&T Cooperation

CALL FOR PROPOSALS

Under the aegis of an inter-governmental Agreement on Scientific & Technological Cooperation, the Department of Science & Technology and the Chinese Ministry of Science & Technology have jointly agreed to support scientific visits for (i) senior scientists/researchers under a PEEP Programme (Project Exploration Expert Exchange Programme) with an objective to have a comprehensive assessment of the synergy and scientific potential available with Chinese organisations and institutions, and (ii) scientists and researchers to undertake study tour to specified Chinese research institutions based on their interaction with Chinese counterparts in order to enhance scientific contacts and formulate/implement a joint cooperation project in a specific topic of common interest.

2. Proposals are invited under the above two schemes from scientists/researchers working in priority areas of research, among others, in the fields of (i) Biotechnology, (ii) Medical Science and Technology, (iii) Materials Science and Technology, including Advanced Materials, (iv) Earth Sciences, including Natural Disaster reduction, (v) Chemical Science and Technology, (vi) Aeronautics, and (vii) Electronics Hardware and Information Technology, including Software development. Other areas may also be considered, if found suitable.

3. Proposals, in the prescribed format, are required to be submitted to the Department of Science and Technology at the earliest, but not later than 31 August 1999. Application forms may be sought from the Section Officer, International Division, Department of Science and Technology, Technology Bhavan, New Mehrauli Road, New Delhi 110 016, directly by 31 July 1999. For any further clarification please contact Shri C. R. Murty, Director, International Division through E-mail address murtycr@alpha.nic.in.

CURRENT SCIENCE

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http://ces.iisc.ernet.in/curscinew
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Correspondence	Less than 600 words; short letters preferred
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Research News	Less than 2000 words; display items should be restricted to 2 Figures and 1 Table
Scientific Correspondence	Less than 1500 words and 2 display items
General Article	About 4000 words; 6 display items
Review Article/Research Account	About 6000 words; cited references should be limited to 100
Research Article	About 4000 words
Research Communication	About 2000 words
Book Review	Less than 1500 words

Founded: 1932

ISSN: 0011-3891

CURRENT SCIENCE

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25 June 1999

COVER. The lesser dog-faced fruit bat, *Cynopterus brachyotis*, in Kalakad Mundanthurai Tiger Reserve. See page 1542.

Indexed in CURRENT CONTENTS/GEOBASE/CHEMICAL ABSTRACTS

COVER description (1999, **76**, 1408: 10 June 1999) should read: INSAT-2E Very High Resolution Radiometer imagery (visible) of 20 April 1999.

In this issue

An AFM measurement of elastic modulii

A brief report on the design and development of an atomic force microscope at Central Scientific Instruments Organization, Chandigarh had been published by A. D. Kaul *et al.*, earlier in the pages of *Current Science* (1997, **73**, 738). In addition to discussing some aspects of the mechanical and optical design, the authors had given some results on studies related to surface topography of a holographic grating and a micro-machined silicon surface in that publication. The instrument had also been used for scanning a polycarbonate filter surface showing clearly 200 nm diameter perforations and a surfactant on a polymer macromolecule.

In this issue, A. D. Kaul *et al.* (**page 1561**) have reported results of load–depth indentation measurements, using their atomic force microscope. Mechanical properties of materials on nanometer scale can be studied using an atomic force microscope in the indentation mode because of the high lateral and depth resolutions. A phenomenon referred to as 'reverse path effect', an instrumental artefact that affects quantitative measurements is taken care of by the authors by measuring the displacement of the PZT actuator absolutely, by measuring laser Doppler shift. These data have been used to correct force curves. The elastic modulii of pyrolytic graphite, silicone elastomer, mica and gallium arsenide have thus been measured on a nanometer scale.

K. R. Rao

A celebrity antigen

In 1963, Kare Berg and his colleagues at the University of Oslo discovered a fascinating antigen. They had from different human subjects isolated a protein, which carries lipids in blood (lipoprotein) and injected the fraction into rabbits. Then they tested the reactivity of the rabbit antisera to human plasma samples, in search of variant forms of beta lipoproteins in the human population. Interestingly, they found that only one-third of the samples reacted to the rabbit antiserum and that the others did not. Thus, a new antigen was recognized. The lipoprotein-associated antigen was given the name lipoprotein (a) – Lp(a). In the late sixties and early seventies several studies tried to link Lp(a) positivity or negativity with disease states. Later it was shown that nearly all humans have Lp(a) in their blood, in varying amounts.

The interest in Lp(a) increased in 1974 when Berg, Dahlen and Frick reported association between high plasma levels of Lp(a) and coronary heart diseases. A number of case-control studies have confirmed their observation and suggested that Lp(a) may be an independent risk factor for premature cardiovascular disease. The GRIPS study in which individuals were followed for ten years found that Lp(a) is an independent risk factor for coronary artery disease, stroke and peripheral vascular disease. High blood levels of Lp(a) can contribute to accumulation of fat in the blood vessel wall and also enhance formation of blood clots in vessels.

Lp(a) is now identified as a genetic trait that is autosomally transmitted. It is assembled from low-density lipoprotein and apolipoprotein(a). Apolipoprotein(a) is coded by one of the most polymorphic genes known in humans. Variations in the gene are a major determinant of the plasma levels of Lp(a), which differ considerably between individuals and also across populations. The reasons for the large inter-individual and inter-population differences in average Lp(a) levels in plasma are not known.

Much progress has been made in recent years in the understanding of the structural properties of this lipoprotein, factors controlling the expression of the apo(a) gene, its biosynthesis and biology. K. Luthra *et al.* review (**page 1553**) the current knowledge on the biochemical features and clinical significance of Lp(a).

Lp(a) is especially noteworthy for Indians. People from the subcontinent have Lp(a) levels that are higher than the levels seen in white Caucasians. Why this is so is unclear. Also not known are the normal functions of Lp(a), molecular mechanisms underlying differences in genetic Lp(a) trait among human populations and regulation of apo(a) levels.

C. C. Kartha

Aerobic Antarctic bacteria shun oxygen

Strange are the ways by which life forms adapt to the environment. Some like it hot and are happy at temperatures above 100°C and in contrast some like it cold as the cold loving bacteria from Arctic, Antarctica, ocean beds, permafrost regions, etc. During the last decade attempts have been made to culture the extreme thermophiles and understand the molecular basis of adaptation, a task still undone. In comparison, more is known about adaptation of microorganisms to cold temperatures like their ability to sense temperatures and modulate membrane fluidity, ability to transcribe genes at low temperatures, ability to upregulate certain genes at low temperatures and the role of cold stress proteins. But, from time to time we also encounter totally unexpected strategies to counteract or adapt to stress. Loka Bharathi *et al.* (**page 1585**) working on Antarctic bacteria observed that the surface lake waters in Antarctica which have high dissolved oxygen content paradoxically supported higher numbers of anaerobic bacteria than aerobic bacteria. It is suggested that this phenomenon could be a strategy adopted by bacteria to express viability under reducing conditions when the dissolved oxygen in the surrounding waters has high/saturating concentrations of oxygen.

This is an attractive hypothesis and needs to be understood with respect to the molecular basis of cold adaptation.

S. Shivaji

CURRENT SCIENCE

Volume 76 Number 12 25 June 1999

EDITORIAL

Who's afraid of impact factors

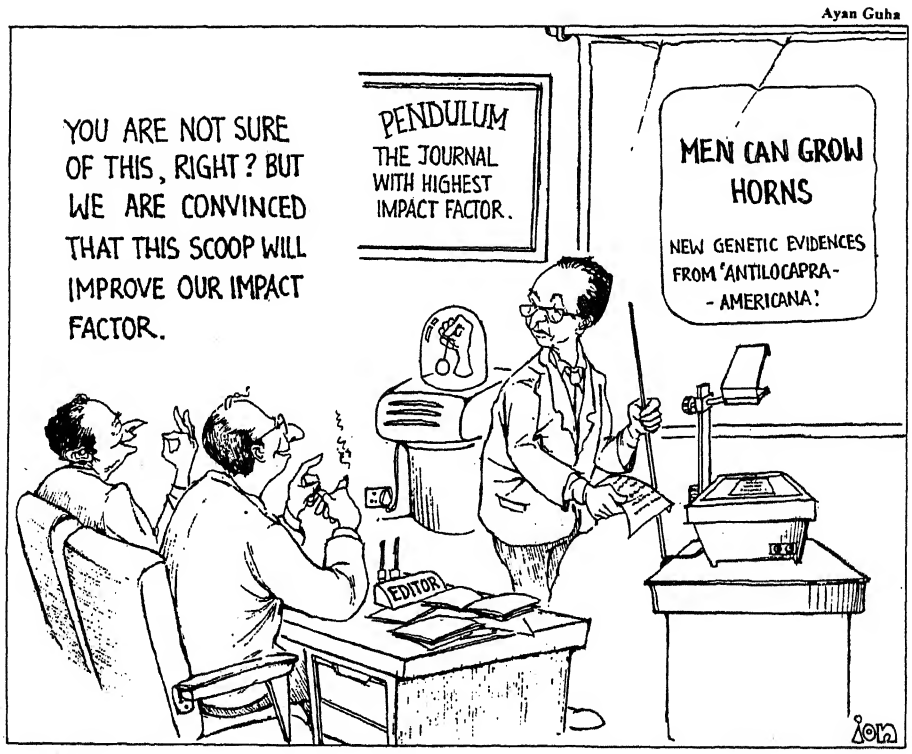
Being an editor of a journal is a difficult (and uncomfortable) business at the best of times. Nowadays, it is even harder thanks to the many precisely-defined measures for assessing journal quality. It is hard for an editor to escape the penetrating question – 'What is the impact factor of your journal'. Presumably, the editors of the glamorous journals (*Nature*, *Science* and *Cell* amongst them) smirk and preen when asked this question. (Indeed some of these journals proudly announce their ranks in colourful advertisements.) Those in charge of lesser known periodicals merely pretend not to have heard the question and heartily wish that their tormentors would one day be appointed as journal editors. It was thus comforting to read that impact factor calculations provoke even the editors of well established, highly regarded professional journals like the *Journal of Bacteriology* (see G. C. Walker, *J. Bacteriol.*, 1999, **181**, 1–3). A recent letter in *Current Science* quotes from the *J. Bacteriol.* editorial and calls attention to the many 'forgotten citations' which are excluded from the calculation of impact factors (J. Gowrishankar, *Curr. Sci.*, 1999, **76**, 1424). This letter further highlights the fact that a science establishment enamoured with impact factors forgets the real purpose of research and 'devotes itself not to making the important measurable but to making the measurable important.' (P. A. Lawrence, *Nature*, 1999, **397**, 487–489). Harsh comments indeed, about a parameter that is now an almost universally accepted measure of a journal's importance.

A little understanding of the formula used for impact factor calculations may help those who wonder why journals cannot easily enhance their ratings. The impact factors announced in 1999 will consider all citations (in journals covered by the *Science Citation Index*, of course) in the years 1997 and 1998, to papers published in the journal in 1995. The 'forgotten citations' are to those made to papers published in the same journal before 1996. Thus, there is an element of immediacy, a touch of fashion and maybe a bandwagon effect in operation, when impact factor counts measure short-term citation rates. Fast moving fields, areas where hundreds (and sometimes thousands) of groups hunt for gold must be well represented on a journal's pages, in order to assure instant citation success. In the recent past there have been many such rapidly moving fields of research, which have dramatically illustrated how 'hot areas' can explode and later subside. The physicists will recall the heydays of high temperature superconductivity; the fullerenes are still fresh in the memory of chemists; while biologists seem to have more long lasting fashions – apoptosis, signal transduction and cell cycle research staying at the top of the charts for some time. The high impact journals (particularly the interdisciplinary ones) must then carefully select the papers that they publish. Editorial decisions can then be guided by perceived importance in an immediate context. The more staid professional journals can hardly subscribe to a scientific fashion show. Not surprisingly, the *J. Bacteriol.* editorial emphasizes that the journal publishes 'truly the best papers in the field and does not bias "decisions by considering the perceived popularity of the topic"'. Since impact factor calculations use short-term citation counts in the numerator and the total number of papers published annually by a journal in the denominator, periodicals which publish a large number of papers are almost always at the bottom of the ranks. The moral, necessarily, is simple – publish fewer papers and try to publish the best (or at least the most 'attractive' papers) in rapidly moving fields in order to enhance impact factors.

In the Indian context, impact factors have assumed a larger-than-life role. All kinds of new (and poorly conceived) indices are being devised, not for evaluating journals but for assessing science and scientists. It is

common to see publication lists being scanned and a scientist's worth assessed by computing an 'average impact factor', based on the journals in which an individual's papers have appeared. In these calculations, the possibility is not even considered that some of the papers published in high impact journals may not be cited, while those in lower impact publications may in fact, have attracted attention. From individuals to institutions is only a short jump. Many analyses of publication profiles currently doing the rounds in scientific circles are characterized by a woeful lack of understanding of the databases used, the parameters derived and most importantly, a complete ignorance of the processes of science. Not surprisingly, ill-informed analysts then draw erroneous conclusions about the scientific health of institutions, departments and individuals. Scientometrics is a powerful tool, but trained practitioners are hard to find. In making decisions about where to publish, prospective authors are sometimes misguided into choosing journals based on 'impact factor criteria'. The simple rule is that papers should be published where they are noticed by others in the field. The discussion on citations and impact factors is never ending but an answer to the question posed in the title is clear – journal editors, of course.

P. Balaram



Why is NET necessary?

According to the recommendations of the Rastogi Committee, set up to study the eligibility criteria for the appointment of lecturers in universities all over India, the NET has become optional. There was always opposition to this criterion. The various state governments devised ways to bypass this criteria in the form of SET/SLET-like tests through an examination. Now that this qualification is made optional, Ph D holders, who could not clear this test will be happy. But it will affect the appointment process badly and needs reconsideration.

The question arises what should be the criteria for the appointment of lecturers? A Ph D degree as a minimum qualification for lecturership sounds good. But we have instances where these degrees are got by unfair means and this has nothing to do with the general competence of the person concerned. People argue that a NET qualification need not necessarily mean a good teacher. Ph D degree too has nothing to do with teaching competence. The NET at least showed the personal competence of those who qualified. In an age when there is stiff competition for every post, this noble job should not be left to fancies of the governing lots in various institutions. It is immaterial whether those who qualify NET are paid scholarship or not, the lecturership eligibility must be retained exclusively. This is the only way by which UGC can exercise some control over the appointment of lecturers in different universities. There may not be consensus over the type and pattern of examination, but some all-India level competitive exam is necessary to see that the appointees are compatible to some national level tests. If there is a need to change the pattern of this examination to reflect the candidate's teaching ability, it will be welcome. It is also advisable that a re-evaluation of the theses be made by a committee at the time of appointments.

VACHASPATI PANDEY

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Proliferation of awards will lower the quality of our basic research

The editorial on 'Promoting Young Scientists' by P. Balaram (*Curr. Sci.*, 1999, **76**, 1059–1060) touches the raw nerve of our scientific community, but without showing the wound visible on a closer look at the ground realities.

With rare exceptions, the lab environment today is such that when one is seriously preoccupied in research activity, not infrequently some others glibly ask, 'Busy working for a Nobel Prize?'. This sarcasm is reserved for such seniors who could, in principle, get the work done by their juniors or research students. That is, the comments are by and for the potential leaders of the scientific community. This is because most of us take to research as a 'job' and, therefore, do not normally feel either the pressure or the urge to work beyond office hours. The image is also consistent with the conclusion of Ashok Khosla (*Curr. Sci.*, 1999, **76**, 1080–1086) on the reasons for our failure to place '... science at the service of the society'.

The unfortunate fact is that we have learnt not to believe that enthusiastic hard work could be driven by intrinsic interest in the research problem at hand. More pertinently for this discussion, we would usually attribute some immediate gain to the 'hectic' activity such as participation in a symposium, or increasing the list of publications for a rise in the hierarchy and its associated perks like recommendation for an award and so on. The Nobel award in such comments serves to epitomize a benefit of a kind different from the normal ones which most scientsts can get without working hard because the threshold of the level of quality for rejection by the peers is exceedingly low – a cursory glance at any symposium proceeding should convince anyone.

Indeed, the cynicism is much deeper. It is implicit that the awardee has some 'Godfather' or 'connection' up there. Rumours pave the way for controversies in newspapers, but spread more through loose talks. Invariably these detractions end up reasserting the unstated but deep faith in our inabilty to do good science as well as in the futility of efforts to arrest the decline. In these private talks, usually, criticality is shunned in favour of juiciness.

Yet the cynicism is not totally unfounded. Going by the Science Citation Index (SCI), our contribution in the global context is poor. It clearly provides an objective basis for the skepticism about the true worth of the awards at least in basic sciences. There is a need to base awards on more transparent and objective grounds than what exist today. Perhaps objective assessment could be achieved by linking the awards to SCI at levels reasonably close to average for National awards in more dynamic scientific communities of the world. However, then there would be fewer winners. The gain, of course, would be enormous by way of return of the missing faith in their worth. But who will bell the cat? Unless the scientific community shows the will to accept the fall-out of the hard steps to be taken through a less flexible set of ethical codes in its routine transactions, the cleansing effort will necessarily end

up as yet another boot-strapping excercise.

An important aspect the editorial leaves out may place the role of awards in research activity in its proper perspective. The pleasure of research is primarily the excitement when the right solution flashes in the mind or gradually emerges through an arduous journey to a finale, punctuated often with failures. This act of creation accomplishes its own reward whose worth cannot be matched by any award. Unfortunately, our science education does not encourage readings which expose lives and minds of great scientists. No wonder that the best of our students consider receiving an award as the ultimate index of excellence. Only a lucky few may encounter a teacher or someone nearer who sensitizes them to the real source of pleasure that drove many scientists even to risk their lives or face social stigma, and not seek awards greedily as we tend to do.

Most present-day leaders of our scientific community grew up with an attitude towards awards that must be unlearnt now if we are serious about the change. I quote from the twenty-two-year-old narration of V.S. Naipaul, now a classic, *India: A Wounded Civilization*. Since obviously, unless corrected, it will also be passed on to the next generation in a more entrenched state, I leave it to the reader to gauge the depth and the gravity of the chronic problem we are afflicted with:

'India grieved for the scientist Har Govind Khorana, who, as an American citizen, won a Nobel Prize in medicine for United States a few years ago. India invited him back and feted him; but what was most important about him was ignored. "We would do everything for Khorana," one of India's best journalists said, "except do him the honor of discussing his work". The work, the labour, the assessment of the labour: it was somehow that would occur elsewhere, outside India.'

An award to a young researcher who is not kindled by the genuine spirit of enquiry could snow-ball for the rest of his/her career if it goes into his/her head; getting awards may become the only objective. With our penchant for politicking and manipulation, and with no effective checks from the mute scientific community, such efforts could succeed in spite of some half-hearted resistances until the practice is accepted by all – so that the only way of getting awards ultimately may be through the back door!

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Olive Ridleys in Orissa: Further comments

With reference to our article 'The Olive Ridley sea turtle (*Lepidochelys olivacea*) in Orissa: The urgent need for an intensive and integrated conservation programme' in *Current Science* (1998, **75**, 1323–1328) we would like to make a few additional points and clarify certain issues that appear to have been misunderstood.

1. We are happy that, after a gap of two years, mass nesting (arribada) took place at Gahirmatha, the major mass nesting site in Orissa. Nesting took place primarily on a 2 km island that is a fragment of the island that broke away from the mainland in 1989 after a cyclonic storm. It is estimated that 210,000 to 250,000 turtles nested during the last week of March on the two islands, Nasi 1 and Nasi 2, mainly the latter. Nesting also occurred on the mainland beach and a new area near Barunei mouth, 30 km south of Gahirmatha, with 8000 turtles nesting in the second week of March and 20,000 turtles nesting on 21–22 April.

However, the mortality figures continued to be high (10,000 dead turtles on the Orissa coast) despite the efforts of the Orissa Forest and Fisheries Department, Government of India and NGO initiatives such as Operation Kachhapa. The absence of mass nesting over the past two years may not be related to high turtle mortalities and therefore, the occurrence of mass nesting should not be taken as a sign that all is well with the turtle population. The changing geomorphology of the Gahirmatha coast may have rendered the beaches unsuitable for nesting. The islands (Nasi 1 and Nasi 2) on which the turtles currently nest are two fragments of the island (Ekakulanasi) that broke away from the mainland in 1989. A substantial proportion of nesting occurs on Nasi 2, the northern fragment, which this year has come into contact with the Outer Wheeler island, where the Defence Research and Development Organization (DRDO) has its missile testing range. Nasi 2 is only 2 km long and only 50–100 m wide throughout its length. The island is inundated during spring tide and a large proportion of the eggs are expected to be lost this year (at the time of writing this piece, field personnel estimated the loss at 80% of the eggs due to inundation and erosion). It is possible that if this beach becomes completely unsuitable for nesting, the turtles will eventually be forced to nest elsewhere. However, it they continue to die at the rate of ten to twenty thousand turtles a year due to trawling mortalities, even this large population will soon become extinct.

The arribada gives us hope, but one should approach the conservation and management of the Orissa turtles with renewed vigour and implement the following measures:

(i) Protection of coastline and offshore waters by monitoring and patrolling key breeding and nesting areas.
(ii) Protected Area status for other nesting beaches at Barunei, Devi River Mouth and Rushikulya.
(iii) Involvement of local fishermen in the conservation programme. We would like to reiterate here that the key to long-term conservation

on the Orissa coast lies in empowering the artisanal fishing community and helping them regain their livelihoods and mobilizing them for the conservation of turtles.

2. We would also like to clarify the positive role played by DRDO in the conservation of the Olive Ridleys. DRDO's missile testing range is on an island adjacent to the major nesting beach (Nasi 2) at Gahirmatha. Over the past three years, DRDO has been meticulous in keeping their lights off during the turtle season. They have also extended assistance to WII researchers working in the area, who have their base on Long Wheeler island, which belongs to DRDO. DRDO has also cooperated by postponing their missile tests until after the turtle nesting season. Further, DRDO's ban on any unauthorized entry into a 6 km radius area around their missile testing range on Outer Wheeler Island would be extremely useful in keeping trawlers away from the breeding congregation. Other organizations (such as Jayashree Chemicals Ltd, Ganjam, near Rushikulya beach) have also cooperated by switching off their lights during the nesting season. It is by cooperation with organizations and individuals who work and live along the coast that we can find a solution for sea turtle conservation.

In conclusion, the occurrence of mass nesting of Olive Ridleys at Gahirmatha in March 1999 was a relief for turtle conservationists. However, turtle mortalities continued to be high despite the efforts of conservation groups and the Forest Department. The various agencies working for the conservation of turtles in Orissa should learn from mistakes of 1999 and work towards ensuring that mortality is substantially reduced in the years to come and ensure that offshore breeding waters and the mass nesting beaches get some measure of permanent protection in the future, particularly during October to May.

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NEWS

Probing fundamental problems with lasers and cold atoms: An Indo-French workshop

The study of the fundamental building blocks of nature is at a turning point. The standard model of particle physics, while highly successful, is destined to undergo several modifications and refinements in the coming decades. Apart from a strong indication from neutrino physics, there are several foundational aspects, including compatibility with gravity, which need to be addressed. The experimental clues required to make progress in this field are expected mainly from three fronts. (a) accelerator-based experiments, which can address some of the issues directly, such as determination of particle mass spectra and direct detection of new particles; (b) cosmology and astrophysics; there are indirect deductions concerning important issues in particle physics (e.g. neutrino physics, unification physics etc.) derived from various astrophysical phenomena coupled with observations on the evolution of the Universe; and (c) high-precision, low-energy, laboratory experiments on a small scale, without the use of accelerators. These non-accelerator particle physics (NAPP) experiments probe particle physics aspects at very low energies, with great precision, to be able to make important statements about phenomena at high energies.

These high precision experiments are driven by novel ideas connecting up the world of high energies to that of very low energies by recognizing phenomena that necessarily involve low energy consequences of high-energy phenomena. Classic examples are proton decay as a consequence of grand unification physics and parity violation in atoms as a consequence of weak interaction between electrons and nucleus.

Significant Indian contributions in NAPP-based experiments were made during the golden era of cosmic ray research. In fact, it is this effort which later laid the foundations for the current accelerator-based research by Indian physicists at international accelerator facilities. Observational high-energy astrophysics is now limited to gamma-ray observations and some air shower experiments. The potential inherent in NAPP experiments in the laboratory to probe fundamental issues which may not be even possible to be addressed using accelerators is yet to be widely recognized and practiced in Indian laboratories.

Recent advances in precision measurements in atomic systems, laser cooling and trapping of atoms, trapping of single ions in electromagnetic traps, atomic interferometry, quantum photon interferometry, ultrasensitive torsion balances, low temperature detectors, etc. are expected to contribute significantly to studies in aspects of particle physics in the next decade. With this in mind, and with a view to starting new activities in non-accelerator physics with tools from atomic physics, optics and other techniques, a discussion meeting was organized in Bangalore by R. Cowsik in 1992. Subsequently a major international conference (ICNAPP 94) was also held in 1994 at the Indian Institute of Astrophysics (IIA) in Bangalore.

During the IX plan, the idea of a centre or laboratory for NAPP activity gained momentum, and the need for a

nodal laboratory where research and training could be carried out in this fertile field has been generally welcomed by many senior physicists. One of the planned responsibilities of such a laboratory was to bring together physicists from India and the rest of the world for discussing frontier areas, which have substantial potential to probe fundamental problems in physics. An Indo-French workshop on 'Probing fundamental problems with lasers and cold atoms' was organized to catalyse the necessary awareness and interactions in one of the most active areas of current physics research, namely laser manipulation, cooling and trapping of atoms and ions. The workshop attracted a large number of participants from India and France and has been recognized as the pioneering meeting which brought together a large number of physicists interested in these fields.

The workshop was sponsored by the Indo-French Centre for Promotion of Advanced Research. Several institutions in Bangalore helped in its organization. The French government and embassy provided necessary support and encouragement to enable the participation of Nobel-Laureate Cohen-Tannoudji in the workshop. Inaugurated on 3 January 1999 by B. V. Sreekantan, who has made important contributions in cosmic ray-based particle physics research, the workshop held at the library hall of IIA, continued at a hectic pace with large number of lectures, discussions, and interactions till 9 January.

The study of atoms, of light and their mutual interactions has been among the most productive and influential areas of modern physical research. Apart from being the source for fundamental theories like quantum mechanics, it has served as an effective testing ground for physical theories through possibilities for high precision measurements as well. The development of lasers and the quest for ultra-high precision spectroscopy resulted in developments starting in the mid-seventies culminating in recent spectacular achievements in manipulating atoms with laser light.

Many of these fundamental studies and applications benefit from freezing the natural thermal motion of gaseous atoms. Tremendous progress has been made in achieving this goal over the past two decades or so, exploiting the fact that laser light could be used to manipulate atomic motion. Laser light can be employed to stop a moving atom, or to push an atom in a desired direction. Resulting slow beams, fountains and dense clouds of atoms serve as convenient physical systems where ultra-high precision measurements could be made. Atoms bathed in a multitude of laser beams can get trapped at the intersection of the beams forming a dense, ultra-cold (nearly zero motion) cloud of gas. This cloud can be manipulated in various ways to further freeze out motion of individual atoms leading to spectacular physical phenomena like the Bose–Einstein condensation.

A beam of atoms can be focussed on to a fine spot by pushing with light, and this has become an important tool for high-resolution lithography. The possibilities of using ultra-cold atoms are enormous and applications span from precision measurements of physical quantities and fundamental constants to biology and medicine.

It is also possible to trap a single-charged atom (an ion) and then freeze its motion using laser light. Cold atoms and ions also serve as ideal systems of atomic clocks of unprecedented precision exceeding a part in 10^{17}.

Some of the pioneering ideas which led to these remarkable developments in the last two decades were conceived and elaborated by French physicists, under the leadership of Claude Cohen-Tannoudji who was awarded the Nobel Prize in 1997. Michèle Leduc, the present director of the Laboratoire Kastler Brossel (LKB), Ecole Normale Supérieure, Paris where many of these discoveries were made, and Ramanath Cowsik, who has been actively advocating the need for a new era of NAPP activity in the country, were the major motivating force behind the organization of the workshop, with a clear idea to foster collaborative research in this important area.

IIA has plans to probe the fundamental issues like parity and time reversal symmetry in particle physics and the nature of electromagnetic vacuum, in its laboratories in the near future. There are other institutes like IISc, and RRI in Bangalore, BARC, Mumbai, CAT, Indore, and NPL, Delhi where laser cooling experiments have been started with goals ranging from observation of Bose–Einstein condensation to realization of high precision atomic clocks. The expertise on laser cooling of atoms and ions is only starting to get developed in India. However there exists a rich research tradition in modern optics and laser physics. Therefore it was gratifying to bring together about 130 physicists from all parts of India, representing all the major institutions interested in modern atomic physics and optics for this week-long workshop.

The scientific sessions started with an overview lecture by R. Cowsik on the paradigm of non-accelerator particle physics in which he outlined the see-saw-like connections between high energy phenomena and low energy probes. Martial Ducloy of the University of Paris Nord gave an introductory lecture on Doppler-free spectroscopy, a subject that motivated some of the most remarkable developments in laser cooling techniques. This was followed by a more specific lecture on Doppler-free spectroscopy of alkali atoms, and in particular of sodium by K. K. Sharma (IIT, Kanpur). Cohen-Tannoudji's lecture on subrecoil laser cooling and Levy statistics opened the fascinating world of laser cooling and its techniques. The pioneer was the maestro and also the teacher.

Claude Boccara (ESPCI, Paris) lectured on the VIRGO gravity wave project, especially on the amazingly tight optical tolerances required to achieve the goals and on methods to realize and measure these, F. Vedel on trapping and cooling of ions, including single ions for applications like ultrastable atomic clocks, Bhanu P. Das (IIA, Bangalore) and Philippe Jacquier (LKB, Paris) on violations of the fundamental discrete symmetries, especially parity violation and related effects in atomic systems. Parity violation experiments are of special interest to Indian physicists working in this area since there are two projects starting to probe violations of discrete symmetries in atomic systems. Also, there is a body of leading theoretical work on parity violation, anapole moment and the electric dipole moment in atomic systems by the group of Bhanu Das at IIA. Angom Dileep Singh represented this group and gave a talk on calculations of EDM and on some new experiments on EDM employing laser-cooled ytterbium atoms. Philippe

Jacquier also talked in detail about the ongoing experiment on parity violation pioneered and pursued by Helene Bouchiat – the mother of parity violation experiments in atoms – at the Kastler Brossel laboratory. The experiment uses a pump probe scheme and amplification of the weak signal employing stimulated emission. Pierre Glorieux (Université de Lille) talked about chaotic dynamics applied to lasers, cavities and coupled laser systems. He outlined how experiments using relatively cheap semiconductor and fibre lasers have revealed many interesting aspects of dynamics of light in cavities, which are of importance in applications. Fabien Bretenaker (Université de Rennes) talked about laser physics at Rennes, and gave a detailed account of research on the fundamental linewidth of lasers in non-Hermitian cavity configurations. Alain Aspect gave a fascinating lecture on interaction of cold atoms with evanescent light fields and its applications to the study of short-range interactions like the Van der Waals forces. Martial Ducloy reviewed the extensive work done at the University of Paris Nord on the study of modification of optical properties of atoms interacting with light near dielectric surfaces. The phenomena studied include Mie resonances of dielectric spheres and giant atom-dielectric forces with possibilities leading to atom trapping.

Nonlinear optics has a strong base in India, both in experiments and in theory. K. Rustagi (CAT, Indore) reviewed collective effects in nonlinear optical response and G. S. Agarwal (PRL, Ahmedabad) presented an overview on coherent control of resonant nonlinear optical processes. Rupamanjari Ghosh (JNU, Delhi) discussed methods of generating non-classical states of light and their properties in a unified approach. R. R. Puri (BARC, Mumbai) and N. Nayak (SNBCBS, Calcutta) described their work on micromasers, an important tool in experimental nonlinear optics and atomic physics especially in issues involving interaction of small number of photons with single atoms in ultra-high finesse cavities. S. Dutta Gupta (University of Hyderabad) talked of microspheres as cavities sustaining whispering gallery modes of very high Q values. Such cavities have become an important tool for probing issues concerning cavity quantum electrodynamics.

Interaction of intense laser fields with matter is an emerging field of experimental research in India and an Indo-French workshop on this topic is being planned by practitioners of this field here. G. Ravindra Kumar (TIFR, Mumbai) reviewed the topic and described several experiments in which intense light fields exceeding terra watts of power modify atomic behaviour beyond levels not accessible by the perturbation approach. One can foresee emergence of new physics when such light fields are directed on single trapped ions or on ultra-cold atoms.

Precision atomic clocks are approaching the stability shown by pulsar clocks and the next generation technology involves ultra-cold clouds of atoms like cesium in zero gravity environment in a spacecraft or space station. The first effort in this direction, which is more or less tested and perfected in the laboratory, is called PHARAO, an experiment involving many French groups. Noël Dimarq (Université Paris-Sud, Orsay) described the compact, space compatible design and its implementation, and also discussed applications of cold atoms for the measurement of time, and gravitational and inertial accelerations.

Bose–Einstein condensation in dilute atomic gases and its applications were in focus in many talks. Coherent Josephson tunneling between coupled BEC clouds was discussed by Srikanth Raghavan (University of Rochester) and Subhasis Sinha (IMSc, Chennai) discussed collective excitations in the BEC. Marc Olivier Mews (LKB, Paris) described the experimental techniques involved in achieving BEC, highlighting the example of alkali atoms.

Laser cooling of atoms and ions is perfected to a stage whence these systems are employed in many experiments to probe foundational aspects, especially of quantum physics. The gedanken era has been converted to an era of realizable experiments through these advances, and naturally there were many talks on foundational aspects of quantum physics, spiced up by the presence and active participation of Alain Aspect (Institut d'Optique, Orsay). N. D. Hari Dass (IMSc, Chennai) talked about possibilities of using trapped ions for tests of quantum gravity and for testing the controversial concept of protective measurements. S. M. Roy (TIFR, Mumbai) discussed a causal quantum theory which is more realistic than the de Broglie–Bohm theory. The new theory has experimentally testable predictions, which could be accessible with the new technology involving cold atoms and light. Anu Venugopalan (PRL, Ahmedabad) talked about how mesoscopic superpositions evolve into classical-like states through decoherence with an example involving atoms in cavities. Apoorva Patel (IISc, Bangalore) gave an overview of the emerging field of quantum computation where advances in trapped ion technology are playing a major role.

The Indian ambitions in the area of laser cooling and trapping were represented by several institutions including BARC, IISc, RRI, NPL, CAT and IIA. S. A. Ahmed, B. N. Jagtap and Pushpa Rao (BARC, Mumbai) outlined the research planned by BARC in the areas of laser cooling of neutral atoms and trapping and cooling of ions. Vasant Natarajan of IISc, Bangalore talked about an important application of laser cooled atoms – lithography. Santa Chawla (NPL, Delhi) described the NPL programme for building high stability atomic clocks employing laser-cooled cesium atoms. With possibilities of Indo-French collaboration, this important programme of high precision metrology is sure to gain considerable momentum if sufficient funds become available.

There were several talks on ongoing experiments in the frontier areas of laser cooling and trapping. One of the exciting talks was on the attempt to trap and cool fermionic atoms (^{6}Li) to obtain a degenerate fermionic gas of weakly interacting atoms in a trap. Marc Oliver Mewes described the motivations and also the experiments in progress at the Kastler Brossel Laboratory.

Michèle Leduc and Unnikrishnan (TIFR, Mumbai and IIA, Bangalore) described the experiments at ENS, Paris on laser cooling and trapping of helium 4, and indicated the future goals including laser cooling of helium 3, study of cold atom collisions and possibility of Bose–Einstein condensation in helium 4.

One of the major attractions of the workshop was the three public lectures

by the visiting French physicists, all of whom have made pioneering contributions. Claude Cohen-Tannoudji talked about manipulation of atoms by light at the NIAS auditorium, in the Academy Lecture organized by the Indian Academy of Sciences. He was welcomed and introduced as the latest Honorary fellow of the Academy. A day later, the auditorium at RRI overflowed with enthusiastic students and scientists to listen to the inimitable Alain Aspect talking about his famous experiments on the EPR problem and Bell's inequality. The third public lecture was given by Michele Leduc at IIA on an important development in medical imaging of lungs and cavities, a technological breakthrough from her group's extensive research on helium 3. The magnetic resonance imaging technique employing optically spin polarized helium 3 could be a major tool in diagnosis and treatment of lung diseases.

The afternoon on the last day of the workshop was dedicated to a discussion on future collaborative research between Indian and French laboratories. P. G. S. Mony, Director, IFCPAR outlined the procedure for proposing collaborative programmes and offered cooperation and support. Several definite proposals were discussed, and these are expected to be followed up this year. In the coming years several fruitful collaborations between the French and Indian scientists are expected to come up, which would fulfil the goals of the present workshop.

C. S. Unnikrishnan (with help from colleagues at the NAPP laboratory, IIA, Bangalore) Tata Institute of Fundamental Research, Mumbai 400 005, and Indian Institute of Astrophysics, Bangalore 560 034, India

RESEARCH NEWS

Curious or dubious: The story of a hydrocarbon with an exceptionally short C=C bond length

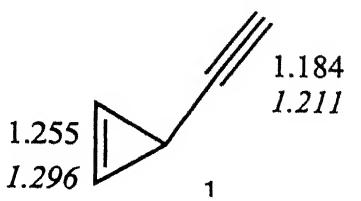
J. Chandrasekhar

One of the greatest achievements in molecular sciences this century has been the accurate determination of structures of a large number of chemical systems. While a variety of spectroscopic and diffraction methods have been used successfully for this purpose, X-ray crystallography has led the way. For the vast majority of chemists, single crystal X-ray structure determination represents the ultimate unimpeachable evidence for the proposed structure. The geometric details obtained through crystallography have also served as the basis for our present understanding of structural chemistry. These data represent an important source of parameterization as well as benchmarks for empirical theoretical models. The quality of quantum chemical procedures, which often use drastic approximations while solving the Schrödinger equation, has also been judged through comparisons of structural predictions with X-ray results.

Interestingly, in recent years, the roles seem to have been reversed. The accuracy of quantum chemical calculations is getting better all the time. And X-ray structure determination is not always devoid of problems, a fact well recognized by the best of professional crystallographers[1]. It is now being suggested that artifacts in experiment may perhaps be identified through comparisons with computational studies. Not a pleasing prospect to many. A recent as yet unresolved controversy provides an example of arguments we may expect between cutting edge theory and experiment.

One always hopes to find something interesting with small ring systems and the rather innocuous hydrocarbon 3-ethynylcyclopropene (**1**) amply fulfills the expectations. The X-ray structure determined at 120 K by Baldridge *et al.*[2] revealed an unusual feature. While all the geometrical parameters were typical of the subunits present in the molecule, the double bond length in the ring was found to be exceptionally short. In fact, the value of 1.255 Å represents the shortest formal double bond ever measured crystallographically in a hydrocarbon.

Acceptor groups are known to reduce the remote bond length in cyclopropyl units, with many examples known from different types of experiments[3]. The elegant analysis of Hoffmann invoking the cyclopropane Walsh orbitals in inducing this structural distortion is textbook material in qualitative MO theory[4]. Even the simplest of theoretical methods reproduce this effect. However, the bond contraction in **1** is of a far greater magnitude. Baldridge *et al.* therefore chose to test what high level *ab initio* theory had to say regarding the C=C length in **1**. Using a variety of



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theoretical methods ranging from Hartree-Fock to correlated procedures and also density functional methods in conjunction with large basis sets including polarization functions, the calculated bond length was consistently larger, being in the range 1.27 to 1.30 Å. The authors concluded that the 'deviation could come from difficulties in approximating the orbital arrangement in **1**'.

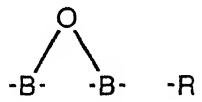
This disturbing conclusion provoked Schleyer and Schaefer to bring to bear considerable computational power to

solve the problem[5]. A sophisticated procedure with the acronym CCSD(T)/TZ2P + f was used, i.e. coupled cluster singles and doubles method including a perturbative triples correction in conjunction with a triple zeta basis set embellished with 2 sets of polarization functions and a set of *f* functions. This level has been shown to reproduce gas phase experimental bond lengths to within 0.008 Å, and the fact was again demonstrated with results for acetylene, methylacetylene and cyclopropene. Geometry optimization yielded a bond length of 1.2957 Å for the double bond in **1**. The key discrepancies between the experimental and *calculated* bond lengths are shown in the structure drawing.

Under these circumstances, it is customary to assume that theory is at fault. The best level is perhaps not good enough. However, this is unlikely in the present instance. The second serious charge against theory is that it refers to the gas phase and direct comparison with the solid state structure is strictly not tenable. It is true that significant differences between gas phase and condensed phase structures have been documented experimentally. A dramatic example involves the complex HCN–BF_3, for which the N–B distance differs by 0.84 Å between the gas phase (microwave) and solid state (X-ray)[6]. However, these differences occur in strongly polar systems with weak bonds. Furthermore, the gas phase structure as well as the changes due to the polar reaction field in the condensed phase are both adequately reproduced through calculations[7]. Strong medium effects are not expected in a nonpolar hydrocarbon like **1**.

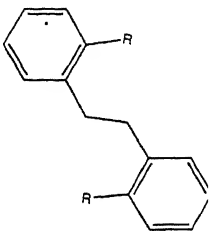
The needle of suspicion should therefore then turn towards experiment. The most common problems associated with X-ray structure determination are errors in space group assignment, errors in atom assignment and the presence of disorder and twinning in crystals. Marsh[8,9] and Parkin[10] have over the years highlighted many instances of incorrect structure determinations resulting from the above causes. Recently, Parkin has pointed out[11] a more subtle phenomenon. The least-squares refinement procedure may converge to a *false minimum.* A dramatic example of two fundamentally different structures which both yield an acceptably low *R* factor and well-behaved displacement parameters is provided in Figure 16 of a recent paper by Murphy *et al.*[11]. However, this artifact is a consequence of the presence of a heavy atom in a polar space group. It appears unlikely that the crystal structure determination of **1** suffers from any of the problems mentioned here. The experimentalists, being seasoned campaigners, cannot be expected to be unaware of these issues. It is worth noting that many of the protagonists in this controversy have been involved in earlier skirmishes involving problems of structure determination. It is perhaps appropriate to recall a few examples.

The first oxadiborairane, **2**, was synthesized and characterized by X-ray



crystallography by Boese and coworkers[12]. Refinement led to a structure with nearly linear CBB units and B–O bond lengths of 1.545 and 1.510 Å. For the parent system, *ab initio* calculations predict a much shorter distance, ca. 1.40 Å. The calculations appear reasonable since the molecule is a formally 2π aromatic system. Boese did manage to get a structure with B–O lengths of 1.38 Å if allowance was made for disorder of the boron and oxygen atoms. However, the *R* factor was higher. Going by conventional wisdom, results were provided only for the structure with long B–O bonds. Subsequent higher level calculations by Schleyer *et al.*[13] confirmed that the B–O bonds had to be shorter than what had been 'observed'. Prompted by the theoretical evidence, Boese went back to the earlier structural model. Although the *R* factor could still not be used to unambiguously distinguish between the two possibilities, he was sufficiently convinced to revise the structure in favour of the one calculated. The paper[13] concludes with a remarkable admonition: 'when their results do not agree with high level *ab initio* calculations, experimentalists are encouraged to consider other possible interpretations of their data if this leads to better accord between theory and experiment'.

It is worth mentioning another debate concerning an unusually short bond for the possible resolution of the problem concerning the bond length in **1**. For over half a century, the central C–C bond in 1,2-diarylethanes (**3**) was thought to have an unusually short length, based on X-ray structures. Jeffrey[14] suggested that the central bond



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3 a R = H
b R = Me
c R = Br

is short in all systems in which two unsaturated groups are separated by three single bonds. However, any orbital interaction involving the flanking π MOs with the central C–C bond can only lead to elongation[15]. This led Winter and coworkers[16] to state that the central bond shortening results from 'a so far unrecognized electronic effect of the π-systems'. The likelihood that the problem may entirely be in the X-ray structure was recognized by Kahr when he redetermined the structure of a derivative **3b** with two innocuous bromine substituents. The central length was 1.54 Å. Clearly, the substituent effect could not be that dramatic as to alter a distal bond length. In a collaborative effort with Siegel *et al.*[17], a convincing case for the presence of an experimental artifact was made through comparison with theory. In contrast to all the crystal structures *ab initio* calculations at the HF/6-31G* level (now considered a modest level) yield a central bond length of 1.54 Å, as normal as can be.

The riddle was solved by Ogawa and coworkers[18] by carrying out the structure determination of **3a** at several temperatures. The central bond length changes from 1.506 Å to the almost normal 1.529 Å as the crystal is cooled from 240 to 100 K. In the related dimethyl derivative **3c**, the distance is nearly normal in the entire temperature range. The results were interpreted in terms of

limited intramolecular motion in the crystal. If the molecule is assumed to vibrate in a direction perpendicular to the plane made by the central four carbon atoms, the average distance between the central carbon atoms will appear to be shorter than the actual value.

The above interpretation is not restricted to 1,2-diarylethanes alone. A similar explanation was invoked to account for the apparently short C=C bond lengths found through X-ray crystallography for stilbenes[19]. In general, molecules with fragments of uneven sizes may not pack uniformly tightly. Some parts may have greater room for large-amplitude motion. This in turn may affect geometrical parameters determined as averages. The same phenomenon may occur in **1**. The molecule has a rigid ring connected to a rod-like ethynyl unit. Movement of the latter much in the manner of a stick-shift in a car could tilt the cyclopropene unit back and forth. The average distance between the carbon atoms would appear shorter as a result. Large amplitude motion of the ethynyl fragment has been demonstrated earlier in a different structure[20]. However, it must be pointed out that the structure analysis of **1** was performed at a fairly low temperature (120 K). Also, Baldridge *et al.* specifically ruled out elongated anisotropic displacement parameters in the direction of the double bond. Nevertheless, a careful examination of the thermal ellipsoids may still be useful.

Before indulging in further speculations on the origins of the failure of theory or experiment, it is perhaps preferable to first identify which one of them is wrong and by how much in the present case. This is best done using alternative experiments, rather than through additional higher level calculations. Neutron diffraction is one possibility. Another approach could be to persuade a synthetic chemist to make derivatives of **1**, preferably with a group which causes the least electronic and steric perturbation and to follow it up with low temperature X-ray structure determination.

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COMMENTARY

Thomas Malthus and sustainable agriculture

Suresh K. Sinha

Thomas Malthus, two centuries ago, wrote '*An essay on the principle of population*'. One of his many conclusions states: 'The existence of a tendency in mankind to increase, if unchecked, beyond the possibility of an adequate supply of food in a limited territory, must at once determine the question as to the natural right of the poor to full support in a state of society where the law of property is recognized'.

Malthus has been described as a multifacet personality, as a demographer, an economist, a politician, a sociologist, and as a moralist. However, he also had a keen perception of agriculture, and in today's terminology the concept of unsustainability is quite important. Both his essays of 1798 and 1830 clearly bring out his concern for agricultural production, labour, and the laws on taxation in the country of produce against free import of the same product from abroad. We recollect here a few of his statements which concern agriculture in the developing societies.

'In the growth of wheat, a vast quantity of seed is unavoidably lost. When it is dibbled instead of being sown in the common way, two peeks of seeds, wheat will yield as large a crop as two bushel, and thus quadruple the proportion of the return to the quantity of seed put into the ground. In *Philosophical Transactions* (1768) an account is

given of an experiment in which, by separating the roots obtained from a single grain of wheat and transplanting them in a favourable soil, a return was obtained of above 500,000 grains. But without referring to peculiar instances and peculiar modes of cultivation, it is known that calculations have often been made, founded on positive experience of the produce of wheat in different soils and countries, cultivated in an ordinary way, and making allowance for all ordinary destruction of seed'.

'Homboldt has collected some estimates of this kind, from which it appears that, the north of Germany, Poland and Sweden, taken generally, produce from five to six grains from one, some fertile lands in France produce fifteen to one; and the good lands in Picardy and the Isle of France, from eight to ten grains for one. Hungary, Croatia, and Slavenia yield from eight to ten grains for one. In the Regno de la Plata, twelve grains from one are produced; near the city of Buenos Aires, sixteen to one; in the northern part of Mexico, seventeen; and in the equinoctial regions of Mexico, twenty four to one'.

There has been considerable progress in the last two centuries in improvement of wheat varieties which today cover large parts of the globe. The seed rate in several parts of the world ranges from 100 to 150 kg ha^{-1}. The highest average yield of wheat in Punjab is 4500 kg ha^{-1} but in many other regions it varies from 1200 kg ha^{-1} to 3000 kg ha^{-1}. If we consider the highest ratio of 1 grain to 24 in production in Mexico, at the time when Malthus wrote his essay, without fertilizer, modern tillage and modern agriculture, it would amount to saying that improvement has not more than doubled.

'Elevated as man is above all other animals by intellectual faculties, it is not to be supposed that the physical laws to which he is subjected should be essentially different from those which are observed to prevail in other parts of animated nature. He may increase slower than most other animals, but food is equally necessary to his support; and if this natural capacity of increase be greater than can be permanently supplied with food from a limited territory, his increase must be constantly retarded by the difficulty of procuring the means of subsistence'.

Malthus did realize that man had the capacity to increase the means of production, thereby in a sense predicting emergence and evolution of new techniques and technology in agriculture. This indeed has happened. But he considered land and its productivity as the potential limiting factors for agricultural production. This is brought out from the following statement.

'The main peculiarity which distinguishes man from other animals in the means of his support is the power which he possesses of very greatly increasing these means. But this power is obviously limited by the scarcity of land – by the great natural barrenness of a very large part of the surface of the earth – and by the decreasing proportion of produce which must necessarily be obtained from the continual addition of capital applied to land already in cultivation.

'It is, however, specifically with this diminishing and limited power of increasing the produce of the soil that we must compare the natural power of mankind to increase ....

'In an endeavour to determine the natural power of mankind to increase as well as their power of increasing the produce of the soil, we can have no other guide than past experience.

'The great check to the increase of plants and animals we know from experience, is the want of room and nourishment were the most abundant'.

He emphasized his point by giving the example of well-peopled countries such as England, France, Italy or Germany which would not be able to meet the food requirement from their land. By using the term well-peopled countries he was only calling them as developed countries in the present context.

'If, setting out from a tolerably well-peopled country such as England, France, Italy or Germany, we were to suppose that by great attention to agriculture, its produce could be permanently increased every twenty-five years by a quantity equal to that which it at present produces, it would be allowing a rate of increase decidedly beyond any probability of realization. The most sanguine cultivators could hardly expect that in the course of the next two hundred years each farm in the country on an average would produce eight times as much food as it produces at present, and still less than this rate of increase could continue so that each farm would produce twenty times as much as present in five hundred years, and forty times as much in one thousand years'.

'If the soil of any extensive well-peopled country were equally divided amongst its inhabitants the check would assume its most obvious and simple form. Perhaps each farm in the well-peopled countries of Europe might allow of one, or even doublings, without much distress, but the absolute impossibility of going on at the same rate is too glaring to escape the most careless thinker. When by extraordinary efforts, provision had been made for four times the number of persons which the land can support at present, what possible hope could there be of doubling the provision in the next twenty five years.

It may be expected, indeed, that in civilized and improved countries, the accumulation of capital, the division of labour, and the invention of machinery will extend the bounds of production; but we know from experience that the effects of these causes, which are quite astonishing in reference to some of the conveniences and luxuries of life, are very much less efficient in producing an increase of food; and although the saving of labour and an improved system of husbandry may be the means of pushing cultivation upon much poorer lands than could otherwise be worked, yet the increased quantity of the necessaries of life so obtained can never be such as to supersede for any length of time, the operation of the preventive and positive checks to population.

If in any country the yearly earnings of the commonest labourers determined, as they always will be, by the state of the demand and the supply of necessaries compared with labour, be not sufficient to bring up in health the largest families, one of the three things stated before must happen; either the prospect of this difficulty will prevent some or delay other marriages; or the diseases arising from bad nourishment will be introduced and the mortality be increased; or the progress of population will be retarded, partly by one cause, and partly by the other.

It is unquestionably true that in no country of the globe have the government, the distribution of property, and the habits of the people been such as to

call forth in the most effective manner the resources of the soil.

It is the laws of nature, therefore, and not to the conduct and institutions of man, that we are to attribute the necessity of a strong check on the natural increase of population'.

The following important points emerge from the above statements, although these statements represent only a few of them:

The availability of land and productivity of soil limit food production; there would be improvement in technology for food production but it cannot match the increase in population; the best example of the productivity potential of wheat at the time Malthus wrote his essay was 1 to 24 seed production. Now the best we have is 100 kg to 5000 kg at field level. In many areas of the world the ratio remains 100 kg to 1500 to 2500 kg ha^{-1}. Therefore, we need to judge our progress more realistically; it was considered important not to favour liberal import of food from other territories to promote own production. This concept is valid even today in view of WTO regime; while Malthus calculated doubling of population in every 25 years, it has actually not happened in many countries. At the time the essay was written the world population was 1 billion. Accordingly, the present world population should have been at least 32 billion (even assuming a doubling time of 40 years) as against 6 billion now. By the same reasoning the population of India since independence should have been 1560 million by now. Fortunately all this has not happened.

One of the many reasons that the population has not reached the level according to the estimates and predictions of Malthus is the conscious control of population despite reduction in death rate. However, it would have been difficult to support the present population if some significant advances in agriculture and food production had not occurred. Let us consider the productivity levels of important crops such as wheat and rice during the times of Malthus and after. The yield of brown rice (husked rice) in Japan in 900 AD was 1 ton per hectare and rose to about 1.8 tons brown rice by the year 1800. The yield of wheat in England was 1.2 tons ha in 1800 AD. Therefore, it is not surprising that Malthus did not expect food production to meet the demand of growing population. Three major scientific steps changed the whole process of food production almost all over the world:

(a) Mendel's laws of inheritance postulated in 1866 were rediscovered in 1900. While earlier than this experiments on plant hybridization were done and segregates (in today's terminology) were obtained, it did not constitute the basis of inheritance. The concept of gene emerged which now is the foundation of genetic engineering and biotechnology. However, the most dramatic effect of the use of genetics in crop yield improvement occurred through the mechanism of heterosis and hybrid vigour. The high yields of maize obtained through this mechanism made crop improvement attractive to entrepreneurs and led to commercialization. This also brought into prominence and focus the importance of germplasm and biodiversity.

(b) Recognition of nutrient requirement and fertilizer use: It was shown by chemists that plants contained many elements in varying qualities. These nutrients must be obtained from soil. This led to the concept of fertilizers and its usage. The chemical process of ammonia production was a major step in this respect which created the whole fertilizer industry.

Realization that plants could mobilize nutrients from the soil only when it was adequately wet and the concept of irrigation which already existed in some form helped in the development of irrigation projects.

There were several famines in India in the nineteenth century. India experienced unprecedented droughts and the consequent famine in 1899–1900 which led the then government to establish the Irrigation Commission in 1901. One of the major recommendations of this commission was to develop irrigation to reduce the impact of drought and the chances of famine through increased production. More area was brought under cultivation, but there really was no significant impact on the productivity of major crops. The productivity of three major crops, wheat, mustard and gram (chickpeas) is given in Figure 1 for the period between 1895 and 1995. Wheat yield did not change until 1965 and that of rape-seed and mustard until 1985. However, a dramatic change in productivity of wheat started from 1970 but there is a slowing down in this crop also of late.

Three factors, genetic, nutrient and water, were independently realized as important for crop improvement and yet until they came together from 1965 a significant change in productivity and production did not occur. Indeed, Malthus had not visualized such an integration of methods or technology for crop production.

(c) The productivity and production of crops has often been reduced by diseases and insect pests. In mid-1930s, pesticides, particularly for insect pest control, were discovered and quickly became a component of agricultural practice. Some of the worst famines such as the one in Ireland and Bengal, India were triggered by plant diseases. Therefore, overcoming plant diseases became a major objective around the world. The genetic control of wheat rust in India was achieved earlier than the arrival of Mexican dwarf (*Rht*-1 and *Rht*-2 genes-based dwarfism along with other traits) wheats to India. A detailed study of the genetics of rust resistance shows that most Indian wheats have an important trait.

However, the insect pest problems continue to cause losses in crop productivity. Recent advances in technology such as genetic engineering have led to significant increases in yield in many crops. Genetic engineering resulting in insect-resistant transgenic plants and crops is a major effort these days. Consequently 38% of the transgenic crops are for making plants resistant against diseases and insect pests. Almost all these resistant crops belong to private sector in most countries with the possible exception of China. Since there is a transfer of genes across organisms, the technology has in some regions raised the question of safety and ethics. For example, the corn produced in USA from transgenic corn was not acceptable in Europe. In developing countries including India such crops can play an important role but the problems of ownership by multinational organizations may become a matter of concern, though it is unlikely that public sector transgenic crops would be seen in the

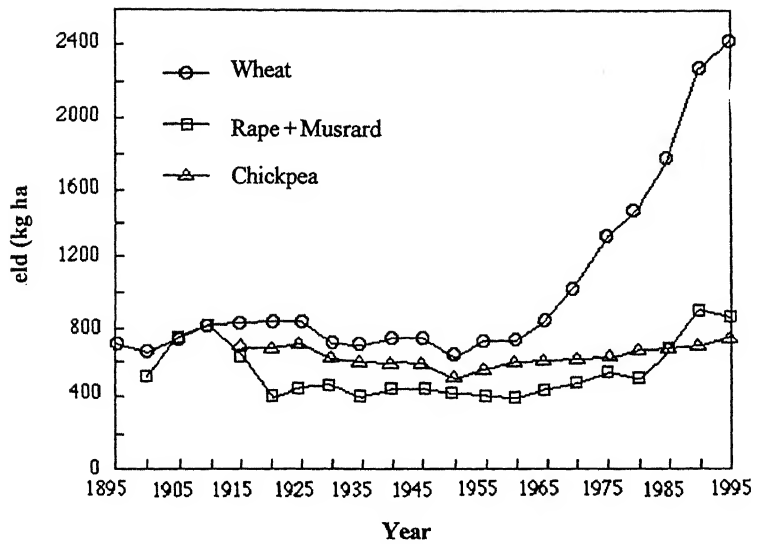

Figure 1. Change in productivity of wheat, rape + mustard and ckickpea in India since 1895 (each point is mean of proceeding 5 years)

farmer's field in the next 5–10 years. Efforts are now on for genetic management of nutrient efficiency. Such technologies help maintain the sustainability of cropping systems. These technologies were not described even in scientific fictions during the times of Malthus.

Future scenario

The future of mankind is still linked with the production and supply of food. The world population is expected to rise from the present 6 billion to 9 billion in the next 50 years. There is already a decline in cultivated area per person and the production of foodgrains per person after stabilization is showing signs of decline. This is because in many parts of the world the actual productivity is far below its potential. Many of these countries do not have access to capital resources and technology to use natural resources for achieving the desired levels of productivity. There are some countries which provide subsidy to farmers to keep land vacant and thus maintain a certain level of production. At the global level there is a possibility to sustain food supply for a regulated population. However, it seems that mankind is moving fast to an era of economic competition and survival of the fittest. Therefore, the question of sustainability has acquired a different perception and meaning to different countries and communities. Malthus talked of ethics and compassion which have been the basic tenets of Indian society, and these would have to be blended with technology to maintain sustainability without imposing segregation.

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OPINION

The rise of the techno-baboo: IT is a brain-sink

Rajesh Kochhar

Computer software is the new pagoda tree which India is shaking vigorously. Earnings from software (SW) exports this year (1998–99) are expected to touch Rs 11,000 cr, up 60% from last year. The rise this year is higher partly due to depreciation of the rupee and the fact that the US has magnanimously agreed to issue entry visas to a larger number of SW professionals than before. One-third of this Rs 11,000 cr comes from a single city, Bangalore, which is home to some 270 firms, big and small, employing more than 80,000 people. The pace of SW growth can be gauged from the fact that barely seven years ago (1991–92), India's SW earnings stood at a paltry Rs 430 cr including Bangalore's Rs 6 cr.

The global SW market is worth $800 bn ($1 bn = Rs 4200 cr), so that India's share of the pie is a minuscule one part in 300. SW exports comprise about 8% of total Indian exports. It is a measure of India's tottering economy that though small as percentage, the SW earnings come as manna from heaven for the beleagured budget-makers.

On the domestic front, the SW effect is discernible in two sectors: motorbike and soft-porn. There has been a spurt in the sale of motorbikes, the entry-level *savari* of the young, ambitious software professionals (that is, the yaspies). This spurt is in sharp contrast to the decline in the sale of scooters, the life-long vehicle of the lower middle class. While the craze for motorbikes is merely a rearrangement within the two-wheeler segment, soft-porn is a new vista. Moneyed but overworked, and away from the loving but intrusive eyes of the fam-

ily, the yaspies seem to be enthusiastic patrons of soft-porn magazines and the colour supplements of newspapers.

Catchphrase

Information technology (IT) is a beautiful catchphrase. Like all good catchphrases, it has the ability to seduce the audience into a mindset of uncriticality. The term technology implies something new or better. The IT India is engaged in is not information technology in the real sense of the term but information tinkering. If IT is equated with designing a new bicycle, India's assignment is no more than fixing tyre punctures.

Big IT companies like Infosys and Satyam have notched up remarkable growth of 100–150% in gross sales and profits over last year. But this growth has been 'almost entirely fuelled by software exports linked to the Y2K [year 2000] syndrome'. When the first generation of computers came up, their memory was extremely small. To save on space, the four digit Gregorian year was abbreviated to the last two digits. Now when the first two digits themselves are going to change, the year must be spelt out in full in all the extant computer programs. It is this glorious donkey work that India has been gainfully employed in.

By definition, the Y2K bonanza would end before the year 2000. India now hopes to turn Europe-wards for lucrative, though more complicated, programming work related to Euro currency conversion. The Indian SW work remains uninspiring and unintellectual. To paraphrase C. V. Raman, recognition (and now money) comes when a proper name becomes an adjective. In Indian SW, no brand names are being built; no value is being added. The profitability of India's SW work does not arise from any intrinsic worth; it comes from the wage differential.

Brain-sink

If we divide Bangalore's export earning of Rs 3500 cr by the 80,000 computerists it is said to employ, we notice that the west is paying $10,000 per Indian professional. In the USA, even a less qualified person would cost at least five times higher. The big Indian SW companies complain how the professionals they send abroad are often lapped up by the companies there. This brings to mind the *mohalla* warfare of yesteryears when the domestic help (comprising mundus and ma'is) was enticed away from one household into another.

In their selfrighteous indignation over brain-drain, the software companies forget that they themselves are acting like a vast national brain-sink. Salaries in the SW market are high; even a fractional dollar salary translates into a tidy rupee packet. And then there is the added attraction of visits and possible employment abroad. The high social value of a programming job more than makes up for its low intellectual worth.

The lucrative but simplistic computer programming contracts that India has been soliciting and executing have trivialized the whole education system. Although there is a mad rush for admission into engineering colleges, the craze for an engineering degree does not mean craze for engineering. Right from the IITs down to institutions run in bicycle sheds, Indian engineering colleges show a wide range in quality. But they all have one thing in common. None of their students wants to work on a shop floor. Everybody wants a ride on the software gravy train.

Recently a student seeking admission into an IIT was matter-of-factly told at the time of counselling not to fuss about the branch he got. In any case he was going to do software. At least a few years ago, IIT Kanpur was planning to close down the aerodynamical engineering. Not because its products were not needed. But because out of the 50 graduates produced, half went abroad, and the remaining went into management/SW. While in the case of the better-quality institutions, the rigorous training is going waste, in the lesser institutions the training itself is being downgraded by the market pulls.

Thanks to the lure of SW, whatever little basic science is offered within engineering colleges is being neglected. There are no takers for the bachelor programmes in basic sciences. For the benefit of students not going to engineering colleges, the degree colleges have started offering courses in computer skills in place of teaching fundamental concepts. We, of our free will, are becoming a nation of techno-baboos. (The old-fashioned spellings are deliberately used to bring to mind the early days of English education in India.)

The techno-baboos now have a new opening, that is, providing remote back-office services to the west. Western airlines, finance companies, hospitals, etc. are setting up offices in India or hiring Indian-based firms to do routine type of semi-manual work. At present, some 25,000 persons are employed in remote services, although the number is expected to go up drastically in coming years. Indian 'data-workers are not rootless part-timers, as their American equivalents may be'. 'Most of those employed in India would be deemed overqualified in the west'.

The colonial science that the British took up in India and in which the natives were employed in a peripheral role was not laboratory science but field science (geography, geology, botany, and even astronomy); it was latitude-driven. More than a century later, the natives are once again being employed by the west. This time their activity is longitude-driven. The working day for the west now lasts a full 24 h, with one difference. The remote office work is got done not by paying overtime, but by one-third the wages.

There are a number of economists and other experts who extol the role of service sector in building up a country's GDP. (Does the burden of the fifth pay commission add to India's GDP?) What is off putting about their analyses is that all the time they are comparing India with Singapore or Hong Kong. India is a country much bigger than either, and far more complex. Also, it is a participative democracy. Its GDP should not only be large but also broad-based. It should cover a vast fraction of the population. This can be achieved only by making the economy knowledge-based.

A handful of people turning in a quick dollar or euro cannot take the economy much further. Bangalore's status as a technopolis does not seem to have done much good to Karnataka as a whole. Perhaps Hyderabad would soon realize this.

For about a century preceding the 1757 battle of Plassey, the British merchants made a lot of money from trade in India. This money went into industrialization of England. The Indian

associates of the British merchants also made a lot of money. But this money went into buying zamindaris and urban properties, and thus became a dead end. In a similar fashion, the SW export earnings are not going into anything progressive; they are simply being frittered away.

India has raised large chunks of loans domestically and internationally. The only way to pay back these loans is to produce wealth vigorously and quickly. The only way to produce wealth is to employ qualified technical people in the task. If these people are placed at the disposal of the industrialized countries at rock-bottom rates in extraneous jobs, who and what would give India strength? It needs to be better known that as far as the unspecified 'non-software electronic exports' are concerned, there was an actual decline of more than 8% in 1997–98 from the previous year, even though the SW exports went up 50%.

There cannot be any objection to India's providing competitive, soiled-collar services to the rest of the world. The trend in fact needs to be encouraged. Globalization, however, does not mean perpetual sunshine in the west and perpetual sunset in India. Globalization means that similarly qualified people anywhere in the world should be more or less similarly employed. Let India's well-regarded manpower take up jobs consistent with its intellect and training. Let the service sector in India flourish on discounted payments but let it not suck in India's trained scientific and technical manpower.

(An earlier version was presented at the UGC-sponsored Seminar on Disturbing Trends in Science Education, Bangalore, 18 February 1999).

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SCIENTIFIC CORRESPONDENCE

Gravity image of India

Regional gravity anomaly maps provide valuable information on the subsurface density distribution, major tectonic and structural lineaments, geodynamic aspects of a plate margin, and structure of the crust and lithosphere, etc. Several papers[1–3] appeared in literature showing the utility of gravity anomaly map of India[4] in geological interpretations. Significance of gravity field in understanding the seismicity and tectonics of the Indian peninsula and the Himalayas[5] and the geodynamic aspects of the Indian plate[6] are also reported. More recently, the importance of gravity anomalies in deciphering the layering of the earth's crust and upper mantle below India[2] is highlighted. Study of gravity trends[3] and synthesis of regional gravity data with available magnetic and seismic information[7] has been done to identify structural provinces[3] and to prepare the tectonic map of the Indian sub-continent[7].

An image of the gravity data digitized earlier[2] is presented in this paper. The purpose is to show the efficacy of geophysical images in displaying the features that cannot be seen readily in a contour map. The colour shaded image of Bouguer gravity anomalies over India is prepared using the GEOSOFT image processing software with both the azimuth and elevation of the sun equal to 45°. However, the commonly available SURFER software or advanced level graphics packages can also be used to create such images. While the basic features of such images would be similar, the application of different graphics packages will normally result in images of different qualities. The image in Figure 1 can be directly correlated with some of the most prominent geological features of India[9]. For example, the Narmada–Son lineament, Godavari and Mahanadi rifts, Aravallis trend and the Eastern Ghats, etc. can be identified by the gravity highs shown by red to yellow colours. The Saurashtra and west Rajasthan block as well as the Shillong plateau are also characterized by high gravity values. Around Mathura (south of Delhi) a gravity high that extends in NE direction as a ridge towards lower Himalayas also appears prominently. A more detailed examination of the map will help to recognize the Cuddapah basin, Vindhyans, and Chattisgarh and Indravati basins – all of them associated with gravity lows shown by green to blue colours.

The features described above provide an obvious correlation with the known geology. A number of interesting features, not reflected in the geological map[9], can be seen in the Dharwar craton. First of all, the Deccan traps, despite being the single largest unit in the geological map of India, do not appear in the gravity image. According to this image map it could be surmised that the Dharwar craton extends northwards up to Tapti river while on the eastern side it is bounded by the Godavari rift. In the northern part of the Dharwar craton there are two strong NW–SE trending parallel gravity high features that run from the western coast to the western margin of the Cuddapah basin. These features might be associated with the rift valleys beneath the Deccan traps reported earlier[10]. Gravity values associated with the trend located in the south also divide the Dharwar craton into northern and southern parts clearly. In the south Dharwar craton, the Clospet granite and the arcuate schist belts in the western part can be identified easily. While the gravity values over the Dharwar craton and the southern granulite terrain show similar magnitudes, a careful examination of the trends clearly shows that the two are separated by the distinct signature of Palghat–Cauvery shear zone.

Among other interesting features, one could recognize two parallel ENE–WSW trends north of Narmada–Son

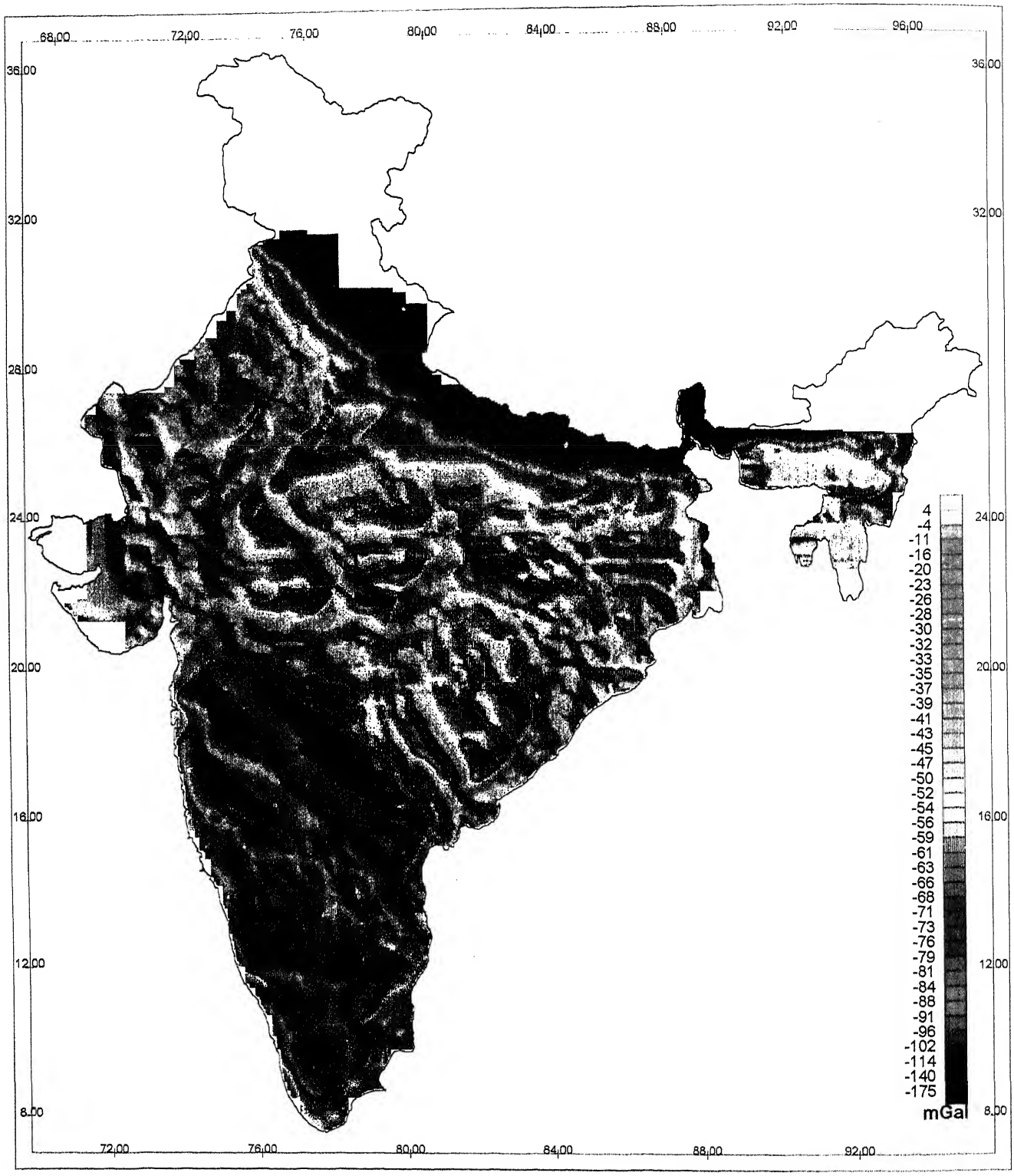

Figure 1. Bouguer gravity image of India illuminated with inclination and azimuth of the sun at 45°.

lineament possibly suggesting the extension of Bundelkhand craton below the alluvials in ENE direction. The NW–SE trends in the northern Dharwar craton, nearly E–W trend of the Narmada–Son lineament, NE–SW trend of the Aravallis, almost N–S trend of the gravity high associated with the west Rajasthan block, and the north-western trend in north-eastern Gujarat, seem to 'radiate' from a region close to the gulf of Cambay. While the aim of this paper is only to emphasize the significance of a geophysical image map, it is worth mentioning that the mega radiating pat-

tern could possibly be used to explain the source for large-scale Deccan volcanism, separation of India from Africa, bolide impact, or plume activity, etc.

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ACKNOWLEDGEMENT. I am grateful to Dr H. K. Gupta, Director, NGRI, for permission to publish this work.

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Poaching, STF-activity and forest loss

Factors driving the forest cover change have become major issues of concern in our attempts to understand the patterns of loss in biodiversity. Occasionally unexpected factors such as certain local-specific social and/or cultural elements are shown to play a very significant role in bringing about the forest cover change. These changes can be quite unobvious and contrary to expectations. We report here one such change in the forest cover in Tamil Nadu that seems to be associated with the increased human activity in the forest. Our purpose is merely to draw attention to an unexpected pattern associated with a specific human activity and not to implicate any specific causal factor.

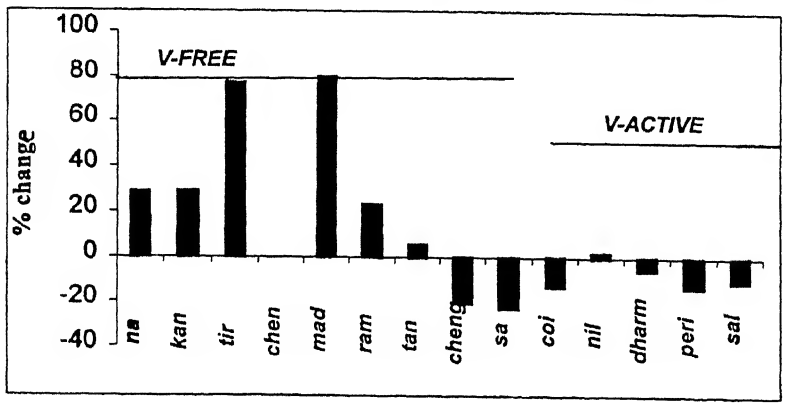

Figure 1. Change in the 'open' forest in the districts associated with Veerappan's activity (V-active) and those free from it (V-free). Note that generally there is a decrease in the 'open' forest of the former area. The districts are: na = North Arcot; kan = Kanyakumari; tir = Tirunelvelli; chen = Chennai; mad = Madurai; ram = Ramanadu; tan = Tanjavur; cheng = Chengulpattu; sa = South Arcot; coi = Coimbatore; nil = Nilgiri; dharm = Dharampuri; peri = Periyar; sal = salem.

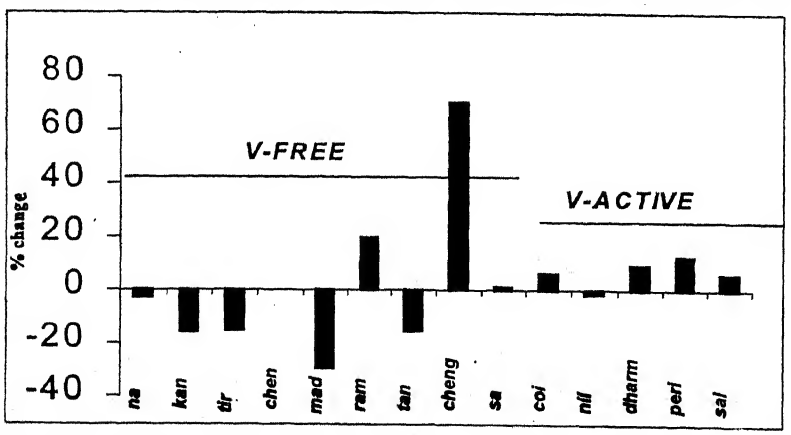

Figure 2. Change in the 'dense' forest in the districts associated with Veerappan's activity (V-active) and those free from it (V-free). Note that generally there is a decrease in the 'dense' forest of the former area. The districts are: na = North Arcot; kan = Kanyakumari; tir = Tirunelvelli; chen = Chennai; mad = Madurai; ram = Ramanadu; tan = Tanjavur; cheng = Chengulpattu; sa = South Arcot; coi = Coimbatore; nil = Nilgiri; dharm = Dharampuri; peri = Periyar; sal = salem.

Poaching is unanimously recognized as one of the important factors for forest loss. In Tamil Nadu (and Karnataka), the Dharmapuri, Periyar, Salem districts and their adjoining areas (Nilgiri and Coimbatore) are well known for the active presence of the notorious poacher Veerappan, and the Special Task Force (STF) has been active in and around these areas almost for the past eight years. Consequently, either because of a mass psycho built-up around these areas or due to severe restrictions laid by the STF for entering them, these forest divisions seem to have received a special protection leading to a significant improvement in the health of the forests. We found that during 1989–1995, when Veerappan's activity came to be highly publicized and the STF was pressed to action, there has been a general improvement in the health of the forest compared to other areas that are free from these factors. The per cent forest under dense cover[1] (> 40% forest cover) increased by 6.36 ± 1.60 while that under open forest (< 40% forest

Table 1. Total forest cover and area under different types of forest in Tamil Nadu district during 1995

District	Recorded forest area*	Area in sq. km			
		Dense	Open	Mangrove	Total
North Arcot	3144.722	1043	1213	0	2256
South Arcot	1125.485	267	722	9	998
Chengalpattu	405.082	89	206	0	295
Coimbatore	1585.517	964	368	0	1332
Dharmapuri	3506.61	1154	1106	0	2260
Kanyakumari	503.14	294	156	0	450
Periyar	2412.239	1090	926	0	2016
Tirunelvelli	1846.336	676	304	0	980
Madras	5.206	0	5	0	5
Madurai	2972.555	1066	1066	0	2132
Nilgiri	1379.342	964	605	0	1569
Ramanathapuram	862.765	186	352	0	538
Salem	1668.874	695	600	0	1295
Tanjavur, Trichy, Pudukottai	1210.459	200	738	–	938

*Source: Division-wise figures received from The Conservator of Forests, Vellore have been reconciled for districts.

cover) decreased by 8.22 ± 2.22 in the Veerappan- and STF-active areas (Figures 1 and 2; Table 1). On the contrary, the areas free from Veerappan and STF-activity recorded an increase in the open forest cover by 22.51 ± 9.52 and negligible change in dense forest cover ($1.34 \pm 6.42\%$). Thus it appears that a significant proportion of the open forest has been converted into dense forest in the Veerappan-active zone while in other areas, there is a substantial conversion of dense forest into open forest.

We do believe that these patterns are not merely by chance. A non-parametric test of direction of changes (increase or decrease eliminating the zero or insignificant changes) also showed that the pattern of change observed in the two categories are significant ($p = 0.03$ for dense and $p = 0.045$ for open forests) and not merely due to random factors. Further, since our comparisons are within a similar state management system, we do not think that the observed pattern can be attributed to any differences in conservation practices; for this reason we have avoided comparison with forests of other states especially Karnataka. While these patterns do suggest a strong influence of the presence of Veerappan and associated STF activity on the health of the forest in an unexpected direction, we do not imply that poaching has its positive impact. It is probable that this is more a local specific process. Hence studies concerning the loss of biodiversity should consider these local factors more seriously than is being done at present.

1. Forest cover is estimated by Survey of India periodically based on remote sensing and ground truthing. The values reported here are from a survey conducted by Survey of India.

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Fish skull from Palana Formation at Hadla–Bhatiyan, District Bikaner, Rajasthan

The present paper reports for the first time a fossil fish skull from the Palana Formation of the Bikaner – Ganganagar Basin in north-western India. Except for some algal and fungal remains[1,2], a rich pollen and spore assemblage[3,4] and a variety of foraminifers[5–8], no mega fossils have been reported from the Palana Formation of Paleocene – Eocene age[9]. The Palana Formation – an important source of lignite in western Rajasthan is characterized by the association of grey clay, grey and greenish-grey to variegated shales, carbonaceous shale, sandstone and lignite.

A well-preserved fish skull was discovered at a depth of about 90 m from the surface when a well was being dug in a field in Hadla–Bhatiyan village (27°46'05":73°03'15") about 45 km south-west of Bikaner town in western Rajasthan (Figure 1). Lithological succession of the study area, based on data from the well at Hadla–Bhatiyan is given in Table 1. The Palana Formation has been observed to rest unconformably over the Badhaura Formation of Permian age, in an exploratory well drilled by the Oil and Natural Gas Corporation at Pugal-1 village. However, the rocks of the Palana Formation show a gradational contact with the overlying rocks of the Marh Formation, Lower Eocene-age, which in turn is disconformably overlain by the Lower to Middle Eocene rocks of the Jogira Formation.

The specimen is the fossilized skull of an Actinopterygian – a fresh water fish (Figure 2 *a* and *b*), at present preserved in the Department of Geology, Faculty of Science, Jai Narain Vyas University, Jodhpur, India. The head measures about 11 cm in length and 8.8 cm in width. Total length of the maxilla and premaxilla is about 5.8 cm. The diameter of the eye is 1.4 cm. Dentary

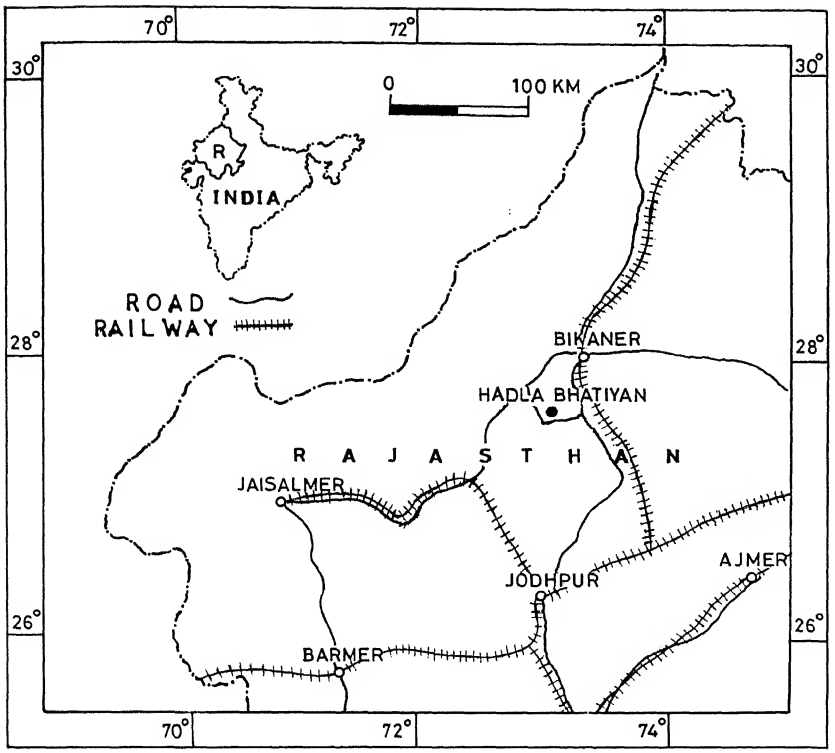

Figure 1. Location map of the study area (R, Rajasthan).

Table 1. Lithological succession at Hadla–Bhatiyan

Formation	Bore hole data (Department of Mines and Geology, Rajasthan)	Data from the well
Recent	Top soil and kankar, 0–6 m	Top soil and kankar, 0–6 m
Marh Formation	Variegated clay and Fuller's earth, 6–63 m	Sandstone, 6–30.5 m Variegated clay, 30.5–62.5 m
Palana Formation	Grey clay, 63–87 m Grey-black shale, 87–100 m Lignite, 100–116.5 m White sandstone, 116.5–128 m Brown and white sandstone, 128–152 m	Black shale, 62.5–85.5 m Lignite with grey shale, 85.5–93.0 m

Table 2. Probable classification of the fossil fish (after Berg[25])

Class	–	TELEOSTOMI
Subclass	–	ACTINOPTERYGII
Division	–	SILURI
Suborder	–	SILUROIDEI
Superfamily	–	Siluroidae
Family	–	Siluridae

has a length of about 5.8 cm. Teeth are sharp and backwardly curved. Lower teeth are larger than the upper ones. Upper teeth are 19 × 2 in number. In the middle part of the dentary the teeth are quite distinct and show an exposed length of 0.5 cm, whereas the unexposed length is about 0.55 cm. Size of the operculum is about 4.6 × 5.2 cm and the subopercular is 3.2 × 1.5 cm in size. Interoperculum lies below the subopercular and posterior to articulum. Preopercular is not well preserved. The opercular series is highly developed with the prominent opercular bone on the lateral side. The mouth opening is large and extends beyond the eyes.

The anatomy of the skull suggests that the fish had a surface feeding character and carnivorous habit. A tentative identification (Table 2) suggests that the fish may be *Silurid* (A. Sahni, personal communication) similar to those reported from the Green River Formation (Eocene) of south-western Wyoming in USA[10].

However, one of the reviewers has suggested that the morphology of the skull and teeth resemble that of *Brachaetus* – an Osteoglossid, known from the Eocene rocks of London[11]. Fresh water fish belonging to the family Osteoglossidae (group Osteoglossomorpha) have been reported from the Lower Eocene sequences of the Himalayan region, particularly, the Subathu Formation[12,13] and also from many parts of peninsular India[14–18]. But there is no report of fossil fish from the Tertiary rocks of north-western Rajasthan.

Fossil fish fauna belonging to the family Osteoglossidae have been reported from Late Cretaceous to Eocene and Oligocene sediments of North America, South America, Africa (Congo, Tanzania and Nigeria), Australia, Denmark, England and Sumatra[19–21].

The present discovery of the fossil Osteoglossid from the Palana Formation of Bikaner in western Rajasthan, supports the fact that Osteoglossids had their origin in India[11,22] contrary to the prevailing view that they had their origin in Africa[23,24].

Further detailed study of the specimen may provide some valuable information about the age of the Palana Lignite and its depositional environment.

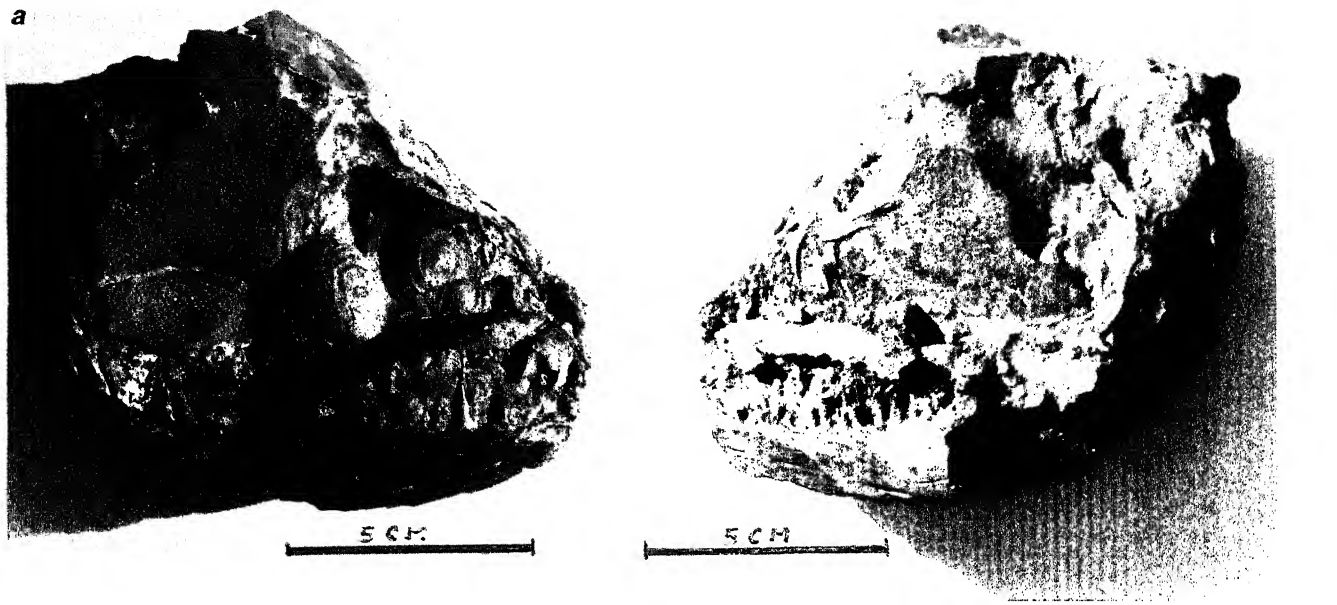

Figure 2 *a*. Right lateral view of the fossilized fish skull. *b*, Left lateral view of the fossilized fish skull.

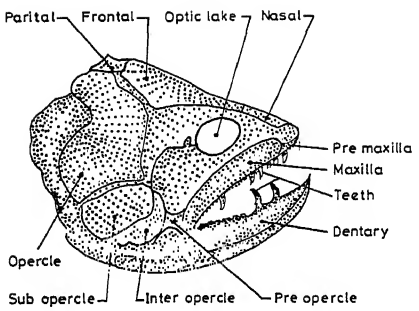

Figure 3. Line diagram of the right lateral view of fossilized skull of the fish, prepared by superimposing it on Figure 2.

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ACKNOWLEDGEMENTS. I thank Prof. Ashok Sahni of Punjab University Chandigarh, Dr K. Kumar of Wadia Institute of Himalayan Geology, Dehradun, and Dr R. S. Rana of Kumao University, Nainital for their help in the identification. Thanks are also due to Dr D. Mohan and Dr A. Purohit of JNV University, Jodhpur, Dr S. Kaushik of Dungar College, Bikaner, and Mr B. S. Mehta and Mr P. S. Shekhawat of Department of Mines and Geology, Rajasthan for their help. Assistance received from Mrs S. Paliwal and Mr B. Paliwal is also gratefully acknowledged.

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Insect remains from Upper Triassic sediments of Satpura Basin, India

This note communicates the first occurrence of three different fossilized microscopic wingless parasitic insects as well as a few fragmented parts of cuticle of different insects along with setae of various lengths (only one fragment which does not belong to these three insects is shown here). The complete insect (Figure 1 *a*) and cuticle of a different insect (Figure 1 *d*) have been recorded from the matrix (yellowish–reddish sandy clay) of overlying Bagra Conglomerates exposed at Khatama caves (30°19': 77°45') Hoshangabad district, Madhya Pradesh (MP) (Figures 2, 3). Two complete insects (Figure 1 *b, c*) have been recorded from the subsurface clay bands exposed in an artesian well-cutting at the village Anhoni (22°38': 78°21') in Chhindwara district, MP (Figures 4, 5). The Denwa Formation (underlying) and Bagra Formation (overlying) are the highest units of the Mahadeva Group. The former, based on the occurrence of labyrinthodont fossil *Mastodonsaurus indicus* Lydekker, 1885 from Denwa beds near Jhirpa, was assigned to Late Triassic age (Keuper)[1]. However, Krishnan[2] considered the presence of *M. indicus* (allied to *Capitosaurus* and *Metapias*) indicative of a Rhaetic age. He viewed upon the age of Denwa beds and Bagra conglomerates as Muschelkalk to Keuper and Rhaetic or/Rhaetic – Lias, respectively[3]. It is suggested that upper part of Lower Triassic to Middle Triassic age for Denwa Formation and Rhaetic? for Bagra[4], palynologically, assigned Carnian to Norian age for Denwa Formation[5]. From the Denwa/Bagra sediments, spore/pollen, dinocysts, fungal remains, trachieds, etc. have also been recorded[6] and assigned, palynologically, the Denwa Formation Norian to Rhaetian in age.

The generalized sequence (as given in ref. 4) is summarized in Table 1 (in part).

The insect (Figure 1 *a*) and cuticle (Figure 1 *d*) have been recorded from matrix of Bagra conglomerates (Sample 1) exposed in the Zamani Nala (Figures 2, 3) near Khatama caves. The Bagra conglomerates have been formed from different kinds of rounded boulders of quartzites, banded jaspers, jasperoid conglomerate, which are loosely cemented by argillaceous matrix (yellowish to reddish sandy clay). The insects (Figure 1 *b, c*) have been recovered from the Denwa clays (Sample 7) from a well-cutting section at the village Anhoni (Figures 4, 5). Denwa clays are always calcareous and often contain numerous calcite nodules. They vary in colour between white and green, red and

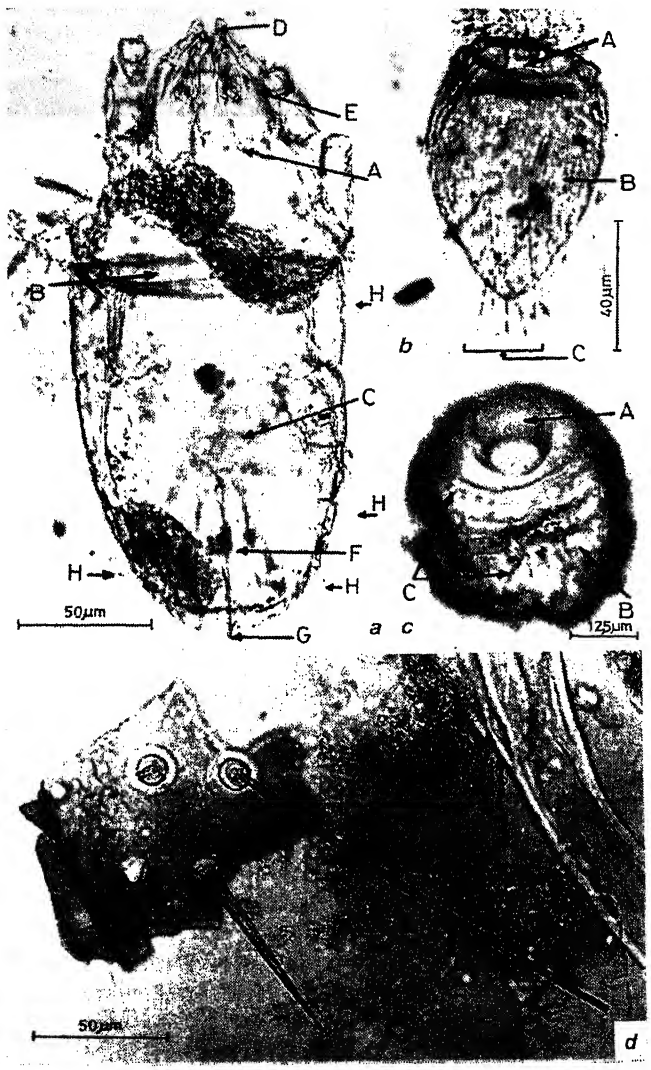

Figure 1. *a*, Complete male fossil insect showing three parts of dorso-ventrally flattened body–head (A), thorax (B) and abdomen (C), saw-like mouth part (D), cone-shaped mouth part (E), genitalia (F), sclerotized part (G), branched setae (H), on body (230 µm long, 105 µm broad). BSIP Sl. no. 12501 (stage coordinates 15 × 99); *b*, Insect having pear-shaped body showing head and thorax fused to form cephalothorax (A) and abdomen (B) with setae (C) (88 µm long and 60 µm broad). BSIP Sl. no. 12053 (SC 17 × 99.5); *c*, Insect having rounded body (43 µm long and 38 µm broad), showing head and thorax fused to form cephalothorax (A) and abdomen (B) with setae (C). BSIP Sl. no. 12054 (SC 28 × 94.5); *d*, Fragmented part of cuticle of an insect showing long (80 µm) and elongate (160 µm) setae with their sockets, and three sockets (14–22 µm) without setae. BSIP Sl. no. 12052 (SC 23 × 103.5).

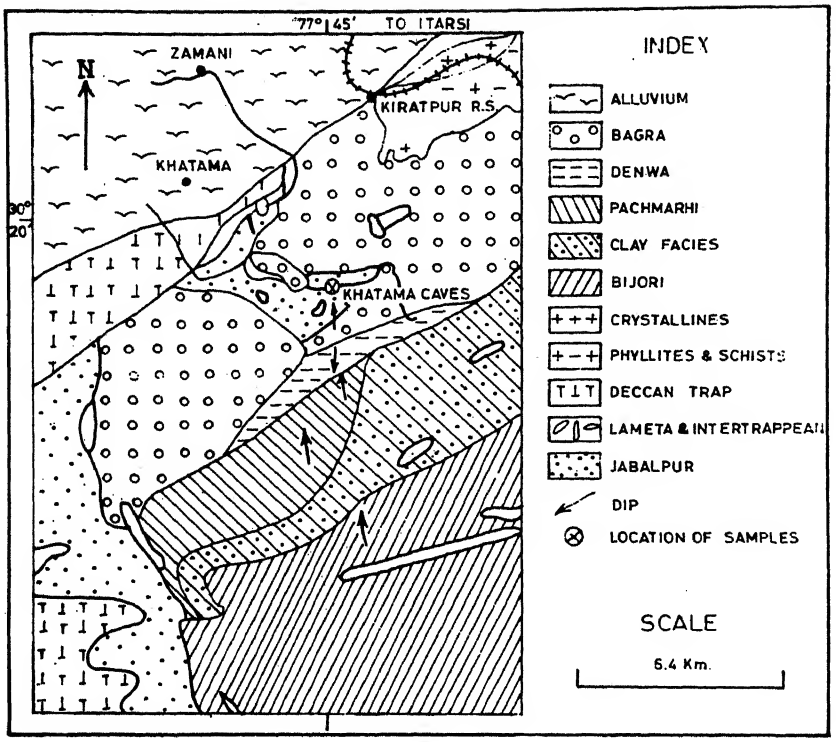

Figure 2. Geological map of the area showing location of the rock samples near Khatama caves. (after Crookshank[1]).

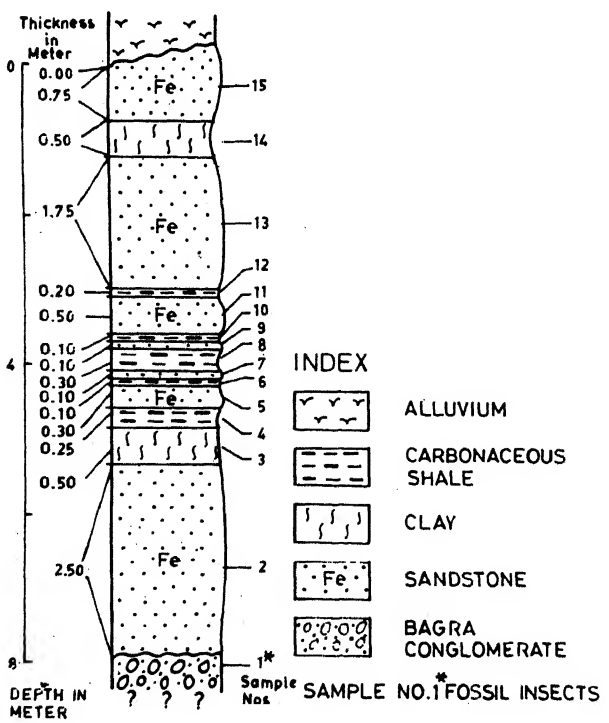

Figure 3. Stratigraphical sequence exposed in Zamani Nala near Khatama caves, Hoshangabad district, MP.

buff. The red ones are the most characteristic. Denwa sandstones are softer and less important. Red jasper pebbles are common.

The complete insect (Figure 1 *a*), is dorso-ventrally flattened, comprising three parts of a typical insect – head (A) prognathus type, thorax (B) and abdomen (C). Its mouth parts are eight in number which are saw-like (D) and cone-shaped (E), to bite and sometime nibble the skin of host. It also shows the presence of external genitalia (F) in the terminal part of the abdomen. Some of its sclerotized part (G) is extended which confirms that this specimen is a male insect. The whole body is mostly decorated with branched setae (H), brownish pigmented.

Locus typicus – Khatama caves, Hoshangabad District, MP.

Stratum typicum – Bagra Formation, Mahadeva Group, Satpura Basin, MP, BSIP, Slide no. 12051.

The insects in Figure 1 *b, c* have a pear-shaped, and rounded-body, respectively, covered with simple setae of various lengths (C). These specimens may be ectoparasitic form on animals, because the presence of different lengths of setae provide them protection from predators. The body is divided into two parts, head and thorax are fused to form a small cephalothorax (A) and the remaining larger part is the abdomen (B).

Locus typicus – Anhoni village, Chhindwara district, MP.

Stratum typicum – Denwa Formation, Mahadeva Group, Satpura Basin, MP. BSIP, Slide no. 12053 (Figure 1 *b*), 12054 (Figure 1 *c*).

Figure 1 *d* is a fragmented part of a cuticle of another insect having simple long (80 µm) to elongate setae (160 µm) with well-defined sockets (12–22 µm). The surface of the cuticle is slightly structured or sculptured.

Locus typicus – Khatama caves, Hoshangabad district, MP.

Stratum typicum – Bagra Formation, Mahadeva Group, Satpura Basin, MP, BSIP, Slide no. 12052.

Fossil lice are not known[7] so far, but the present complete insect seems to be a fossil of mallophagan type of lice as it shows prognathus type of conical head[8], arrangement of setae on the body (chaetotaxy), pigmentation pattern and shape of male genitalia of mallophagan type ectoparasites[9]. Mason and Mar-

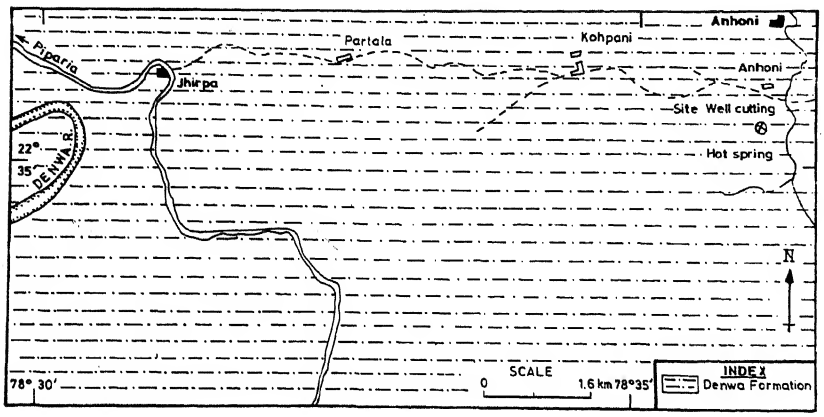

Figure 4. Geological map of Anhoni area showing site of well-cutting (after Raja Rao[4]).

Table 1. Generalized stratigraphic sequence of Satpura Basin

Age	Formation	Lithology (thickness)
Lower Cretaceous	Jabalpur	Massive sandstones with jasper, conglomerates, white clays, red clays, carbonaceous shales and coal lenses (50–100 m)
.....	Unconformity	.....
Rhaetic?	Bagra	Predominantly coarse conglomerates with bands of calcareous sandstones variegated clays, limestone and dolomite (180–240 m)
.....	Unconformity	.....
Upper part of Lower Triassic to Middle Triassic	Denwa	Soft variegated clays interbedded with sandstone bands, conglomeratic at places (about 350 m)
Lower Triassic	Pachmarhi	White coarse grained cross-bedded sandstones with lenses of sub-angular quartz pebbles (about 750 m)
Permian	Bijori	Micaceous, flaggy sandstones and shales at places micaceous(180–250 m)

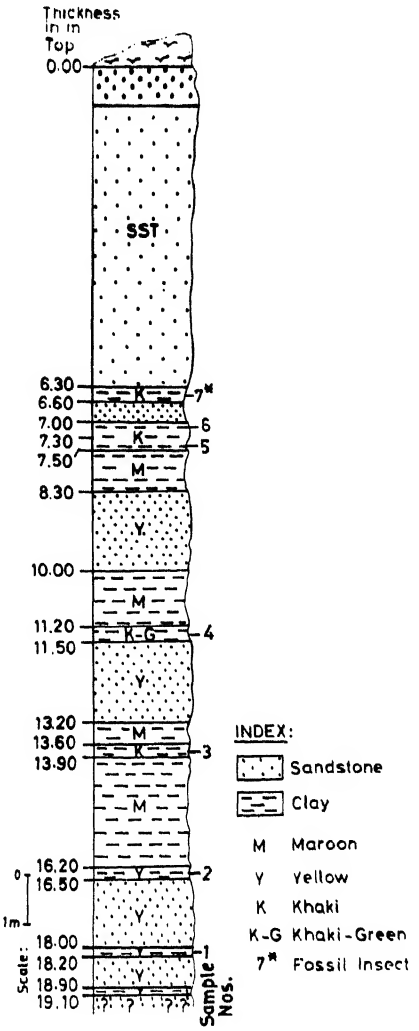

Figure 5. Stratigraphical sequence exposed in a well-cutting at Anhoni, Chhindwara district, MP

shall[10] pointed out that bones of early mammals were present during the early days of Mesozoic era, i.e. about 200 m.y. ago (Late Triassic). Datta and Das[11] have recorded the oldest mammalian fossil tooth from Tiki Formation (Late Triassic) of South Rewa Gondwana Basin, MP, India, which is neighbouring Satpura Basin. So, it must have connections with wandering mammals and it is presumed to be of same age group.

These citations show that the present fossil insects might have evolved during Late Triassic having ectoparasitic habitats on mammals.

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Occurrence of *Cynopterus brachyotis* (Chiroptera: Pteropodidae) in Kalakad Mundanthurai Tiger Reserve, Southern India

During the course of our preliminary survey of bat fauna in Kalakad Mundanthurai Tiger Reserve (KMTR), Tirunelveli, Tamil Nadu (8°44′N; 77°42′E) in October 1998, we collected a few bats of a little known species of the lesser dog-faced fruit bat, *Cynopterus brachyotis* (Chiroptera: Pteropodidae) (Figure 1). Although collection of *C. brachyotis* was not the primary purpose of our survey, the limited data collected on this very poorly studied species in the Indian subcontinent makes the following notes worthy of record.

It is evident from the zoological literature that *C. brachyotis* has a distribution that extends from Sri Lanka to Myanmar, Thailand, Malaysia, Sumatra, Borneo, Sulawesi and Philippines. But it is reported only from a few pockets of Southern India. The provisional localities include Karnataka (Jog Falls, Sirsi and Virajpet), Tamil Nadu (Centre camp near Chinnamanur) and Andhra Pradesh (Balapalli)[1]. This species is commonly found at higher altitudes of the tropical evergreen forests.

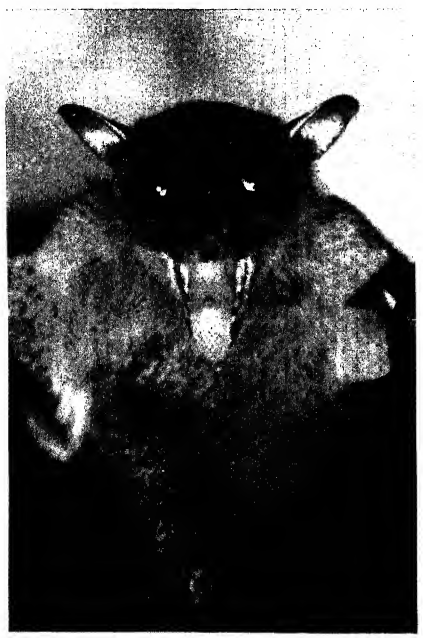

Figure 1. The lesser dog-faced fruit bat, *Cynopterus brachyotis*.

In the present study, we captured a total of 26 bats of *C. brachyotis* by using Japanese mist-net erected at a height of 2 m above the ground level. We did not site any other pteropodid bat such as *Pteropus giganteus* or *Cynopterus sphinx* in the study area. Captures were made on four nights during October 1998, in this tropical wet evergreen forest, at an altitude of 700 to 800 m. The temperature ranged from 15° to 27°C and humidity from 90 to 100% during the study period which were measured using a thermohygrometer (TZ18, Polland). The environment was cloudy with intermittent rainfall. All the mist-netted sites were closer to guava and other fruit trees. Observations of these limited samples suggest that these bats are smaller than *C. sphinx* and adult males have dark brown pelage. *C. brachyotis* possess a mean forearm length of 60.9 mm (SD ± 1.51, range 57.5 to 63.8, $N = 26$), an ear length of 17.7 mm (SD ± 0.62; range 16 to 18.9; $N = 23$) and a body weight of 31.41 g (SD ± 0.95; range 30.0 to 33.2; $N = 20$). Of the 26 bats captured, 8 were males and 18 females. Among the 18 females, 8 were post-lactating, 5 lactating, 2 pregnant and 3 sub-adults suggesting that they were at the end of their breeding cycle during October 1998.

The population of this bat species is distributed in selected areas of the wet evergreen forests and at lower elevations of the hills and is also on the decline due to deforestation, habitat fragmentation, forest fires and human interference. Plant-visiting bats including *C. brachyotis* are of great importance for the maintenance and re-establishment of tropical forest diversity. They play an important role in the tropical forests not only as pollinators and seed dispersers but also as agents in enhancing the availability of food and resources for species such as insectivores and carnivores feeding at other trophic levels[2]. Bats that disperse seeds while foraging can effect recolonization of deforested area. They move seeds over greater distances and wider areas than most other rain forest mammals[3].

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Protective effect of *Picrorhiza kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine-induced hepatitis in rats

Indian ayurvedic medicine claims a lasting cure for human viral hepatitis through oral administration of an extract of *Picrorhiza kurroa* (a member of the family Scrophulariacae). This medicinal plant occurs in alpine Himalayas from Kashmir to Sikkim at altitudes of 2700–4500 m. In traditional medicine, it has also been used to cure heart ailments, lung diseases, abdominal pain, stomach disorders, anaemia and jaundice and to promote secretion of bile[1-3]. Yet there is little documentary evidence regarding its nature, chemistry or action of the therapeutic principle.

Hepatitis induced by D-galactosamine (GalN) shows many metabolic and morphological aberrations in the livers of experimental animals similar to that seen in human viral hepatitis[4]. GalN hepatitis is induced by a multiple step mechanism[5]. Peroxidation of endogenous lipid is a major factor in the cytotoxic action of GalN[6]. A growing body of evidence is emerging which suggests that reactive oxygen-derived radicals play a crucial role in the pathogenesis of GalN hepatitis[7].

Glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species[8]. The cellular tripeptide GSH (L-glutamyl-cysteinyl glycine) exerts protective antioxidant functions through a complex enzyme system including glutathione peroxidase (GPX) and glutathione-S-transferase (GST)[9]. Since the alcoholic extract of dried rhizomes and roots of *P. kurroa* have been reported to contain two electrophilic free radical scavenging principles called picroside I and kutkoside[10,11], we have now attempted to assess the protective effect of *P. kurroa* on mitochondrial glutathione antioxidant system in GalN-induced hepatitis in rats.

The lyophilized ethanolic extract (yield 8.8%) of dried rhizomes and roots of *P. kurroa* (authenticated by Captain Srinivasamurthi Drug Research Institute for Ayurveda, Arumbakkam, Chennai), was supplied by TTK Pharmaceuticals Limited, Chennai. Wistar strain male albino rats weighing 120–150 g were maintained at constant temperature (29 ± 3°C) and light (12L : 12D) controlled room with provision for food (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. Seven days after acclimatization, the animals were divided into four groups of six each. Group I served as the controls. Group II animals received by i.p. injection, 500 mg GalN (dissolved in physiological saline)/kg body wt/ day for two days[2,12]. Group III animals were pre-treated with 50 mg of an alcoholic extract of *P. kurroa* (in distilled water)/kg body wt/day for 10 days by oral intubation and then with GalN (500 mg/kg body wt/day) by i.p. injection for 2 days. Group IV animals were treated with *P. kurroa* alone at the above dosage for 10 days to test for any side-effects.

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected without any anticoagulant and the serum separated was used for the assay of diagnostic marker enzymes such as alanine aminotransferase (ALT) [EC 2.6.1.2][13], aspartate aminotransferase (AST) [EC 2.6.1.1][13], acid phosphatase (ACP) [EC 3.1.3.2][14], alkaline phosphatase (ALP) [EC 3.1.3.1][14], and lactate dehydrogenase (LDH) [EC 1.1.1.27][15]. Liver was excised immediately and washed with chilled physiological saline. Liver mitochondria were isolated by the method of Johnson and Lardy[16].

Mitochondrial protein was estimated by the method of Lowry *et al.*[17]. Lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction as described by Ohkawa *et al.*[18] and the non-enzymatic lipid peroxidation in the presence of promoters such as ascorbic acid, ferrous sulphate ($FeSO_4$) and *t*-butyl hydroperoxide (*t*-BH), 2mM each was studied. Reduced glutathione was measured by the method of Ellman[19]. The method described by Habig *et al.*[20] was followed for the estimation of GST [EC 2.5.1.18] activity and the activity of GPX [EC 1.11.1.9] was assayed by the method of Pagila and Valentine[21].

The results presented here are the mean ± SD of six animals. Level of significance has been evaluated by using Student's *t*-test.

Increased activities of serum ALT, AST, ACP, ALP and LDH are well-known diagnostic indicators of GalN hepatitis. In cases like liver damage with hepatocellular lesions and parenchymal cell necrosis, these enzymes are released from damaged tissues into the blood stream[22]. The present results indicate a marked elevation in the activities of these marker enzymes in serum (Table 1), which is in accordance with previous reports[23-25]. Prior oral administration of *P. kurroa* extract resulted in a significant reduction in the levels of these enzymes towards near normalcy as compared to Group II GalN toxic rats, establishing its hepatoprotective effect.

GalN has been proposed to be hepatotoxic due to its ability to destruct liver cells, possibly by a free radical mechanism[7]. Lipid peroxidation reaction, a type of oxidative degeneration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation[26]. The highly significant elevation in non-enzymatic lipid peroxidation reactions in GalN-induced hepatitis suggests the enhanced susceptibility of the membranes. Significant increases in the levels of mitochondrial lipid peroxides after i.p. administration of GalN have already been reported[27]. GalN-induced hypoglycaemia-related glucose autooxidation[28,29] may also be responsible for the increased generation of free radicals in GalN hepatitis. Our results also suggest that GalN-intoxicated rats may be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate, $FeSO_4$ and *t*-BH (Table 2). Pre-treatment with *P. kurroa* extract in our study significantly prevented this alteration, because of its antioxidant nature[10] against lipid peroxidation induced by GalN[27].

Glutathione has a direct antioxidant function. It functions by reaction with superoxide radicals, peroxy radicals and

Table 1. Activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehyrogenase (LDH) in normal and experimental groups of rats (mean ± SD for six animals in each group)

Group	Group I	Group II	Group III	Group IV
ALT	110.8 ± 9.8	374.3 ± 35.9***	133.4 ± 11.6***	112.9 ± 10.3
AST	85.7 ± 6.2	395.8 ± 36.4***	118.6 ± 9.7***	89.3 ± 6.6
ACP	12.6 ± 1.0	39.7 ± 2.1***	19.2 ± 1.2***	11.8 ± 0.8
ALP	108.7 ± 8.9	263.8 ± 14***	126.2 ± 10.5***	109.2 ± 11.2
LDH	215.6 ± 19.8	398.5 ± 26.4***	228.6 ± 19.6***	212.3 ± 17.4

Group I, Normal controls; Group II, D-galactosamine-intoxicated rats; Group III, *P. kurroa* pre-treated and D-galactosamine-intoxicated rats; Group IV, *P. kurroa* treated normal rats.
Values expressed: ALT, AST, and LDH, μmoles of pyruvate liberated/h/l; ACP and ALP, μmoles of phenol liberated/h/l. As compared with respective controls, i.e. Group II vs Group I, Group III vs Group II: ***$P < 0.001$.

Table 2. Protective effects of *P. kurroa* on mitochondrial glutathione antioxidant system in D-galactosamine-induced hepatitis in rats (mean ± SD of six animals in each group)

Parameter	Group I	Group II	Group III	Group IV
Lipid peroxidation				
Basal	158.14 ± 14.7	269.44 ± 24.3***	182.61 ± 16.5***	163.83 ± 15.2
Ascorbate	324.35 ± 23.8	514.81 ± 40.7***	398.73 ± 29.5***	316.78 ± 25.7
$FeSO_4$	452.83 ± 39.5	686.54 ± 48.9***	524.17 ± 40.9***	445.35 ± 41.4
t-BH	593.46 ± 47.3	864.62 ± 67.2***	678.71 ± 53.4***	602.73 ± 51.7
GSH	38.14 ± 2.78	22.76 ± 1.83***	33.58 ± 2.61***	39.71 ± 2.83
GPX	22.63 ± 2.31	9.84 ± 0.76***	17.58 ± 1.42***	23.21 ± 1.98
GST	2636.33 ± 158.9	1471.24 ± 104.3***	2265.32 ± 129.8***	2594.42 ± 164.3

Values expressed: Lipid peroxides, nmol MDA/mg protein; Glutathione, nmol/g tissue; GPX, nmol of glutathione oxidized/min/mg protein; GST, nmol of CDNB units/min/mg protein. As compared with respective control values, i.e. Group II vs Group I; Group III vs Group II: ***$P < 0.001$.

singlet oxygen, followed by the formation of oxidized glutathione and other disulphides[30]. Depletion of GSH results in enhanced lipid peroxidation[31], and excessive lipid peroxidation can cause increased GSH consumption[26], as observed in the present study (Table 2). Tappel[32] has reported that GSH protects the mitochondrial membrane from the damaging action of lipid peroxide. The oral pre-treatment of *P. kurroa* extract resulted in the elevation of GSH level, which protects against oxidative damage by regulating the redox status of proteins in the cell membrane[33].

GPX, an antioxidant enzyme, offers protection to the mitochondrial membrane from peroxidative damage[34]. A decrease in the activity of GPX makes mitochondria susceptible to GalN-induced damage, which leads to a change in mitochondrial composition and function. Our studies also show decreased GPX activity in Group II GalN toxic rats (Table 2), which is in line with the report by Neihorster *et al.*[35]. GPX and the cellular NADPH-generating mechanism together form a system for removing hydroperoxides from the cell. Prior oral treatment of *P. kurroa* maintained the GPX activity significantly at near normal, as observed in Group III animals.

GST, another scavenging enzyme, binds to many different lipophilic compounds[36]; so it would be expected to bind GalN and act as an enzyme for GSH conjugation reactions. The significant decrease in its activity noted in this study (Table 2) might have been due to the decreased availability of GSH. This is consistent with a reported study[37], which showed a reduction of GST activity in liver. These findings led to the conclusion that GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanism of GalN hepatitis.

Prior oral administration with the alcoholic extract of *P. kurroa* in our study significantly prevented the alterations in the GST activity. It probably did so by counteracting the free radicals due to the presence of two electrophilic substances, picroside I and kutkoside, present in the roots of *P. kurroa*[10,11]. The unpaired electron present in the hydroxyl free radical might have been trapped by these two substances.

Our observations indicate that the pre-treatment with *P. kurroa* prevents GalN-induced hepatitis in rats. This present study suggests a hepatoprotective effect of *P. kurroa* in experimental animals.

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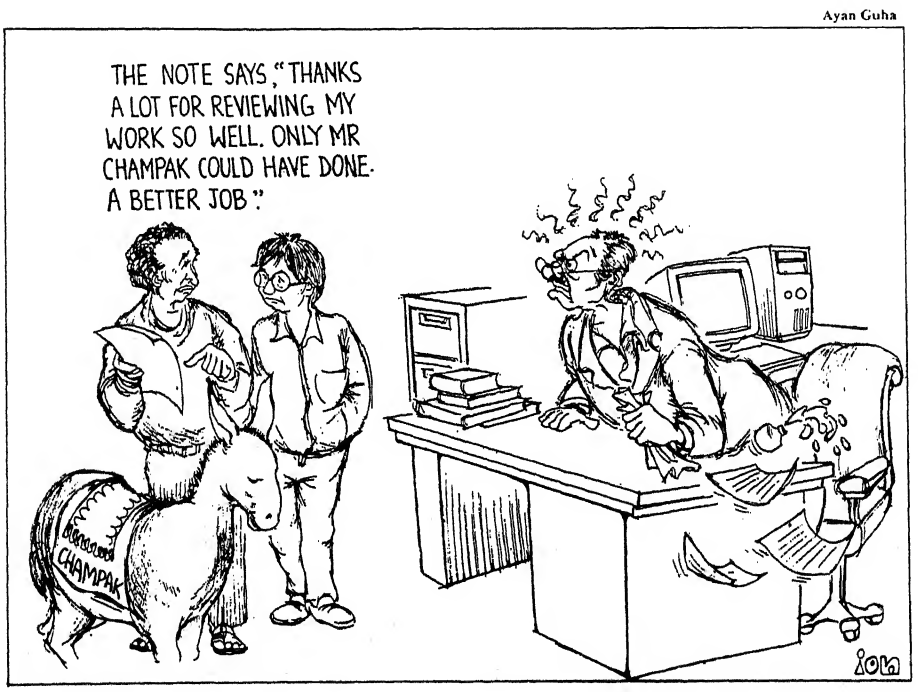

Methane gas: An unconventional energy resource

Alpana Singh and Bhagwan D. Singh

Methane gas in the form of 'coal bed methane' and 'hydrate', probably the last remaining hydrocarbon, is waiting to be exploited as an alternative source of energy. Coal plays the role of source rock as well as reservoir for coal bed methane. Hydrate is a unique chemical compound of methane and water found in deeper sections of ocean floor sediments.

UNITED States and Canada have been exploring methane obtained from coal beds ('coal bed methane') since the early 1970s and 1980s, respectively. Production of coal bed methane for domestic energy needs has grown significantly only in USA. Other coal-producing countries like Australia, China, Russia, Germany, Great Britain, Poland, etc. including India too have paid attention to the exploration of this new resource (now being considered as an economically viable unconventional source of energy) and have initiated several research programmes on different aspects of coal bed methane.

In fact, irrespective of their maturity (rank), organic composition, or nature of occurrence, all coals contain gases, with methane usually as the main constituent (90–97%). However, deep-seated seams (depth around 1000 m or more) containing coal of higher rank (carbon content > 83.5%, R_0 max 0.7% and above) are considered most suitable for commercial extraction of methane. The global coal bed methane reserves are presumed to be several times greater than the total reserves of all the known conventional gas fields.

Around 1969, gas hydrate deposits were first discovered in Russia (Siberian gas fields). As a result, interest in methane from sea floor ('hydrate') began. Natural occurrence of methane hydrate was noticed in other regions of oceanic and terrestrial environments by the 1970s. The encouraging results from discoveries at Blake Ridge (USA) opened up new vistas to consider gas hydrates as a potential source of energy for the future. Currently, countries like Mexico, Japan and India have launched national projects for the exploration of methane hydrate, the most abundant carbon fuel resource.

Conditions of extreme high pressures and cold temperatures favour accumulation of methane hydrates in deep ocean floor sediments, especially along continental margins. It is believed that methane hydrate reserves could possibly hold more fossil fuel energy than is present in conventional oil, gas and coal deposits.

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Coal bed methane

Methane gas is generated during the formation of coal through 'coalification' process of vegetal matter (Figure 1). This can broadly be divided into biochemical and physico-chemical stages of coalification incorporating five successive steps[1]:

Peatification (anaerobic degradation of organic materials in the peat swamp);
Humification (formation of dark coloured humic substances by anaerobic degradation);
Bituminization (generation of hydrocarbons with increase in temperature and pressure);
Debituminization (thermal degradation of matter and generated hydrocarbons); and
Graphitization (formation of graphite).

An excellent summary of the coalification process is given by Levine[2]. Many physical and chemical changes, governed by biological and geological factors, occur during these processes. Whereas darkening in colour and increase in hardness and compactness are the main physical changes, loss in moisture and volatile contents, and increase in carbon content are the main chemical changes. Many acids (humic, fatty, tannin, gallic, etc.) and dry and wet gases (CH_4, CO_2, N_2, N_2O, H_2S, ethane, propane, butane, etc.) are formed during decomposition of the organic matter. All the changes brought about are attributable to the release of –COOH (carboxyl), >C=O (carbonyl), –OH (hydroxyl) and $-OCH_3$ (methoxyl) functional groups from the organic compounds which cause the decomposition of vegetal source matter.

Biochemical stage of coalification, beginning with the accumulation of vegetal matter and terminating at the sub-bituminous stage of coal formation, leads to the formation of a wide range of degradational products – the organo-petrographic entities of coal (termed 'macerals') by the partial oxidation and hydrolytic decomposition of dead vegetal matter accumulated in water-saturated wet lands (basins/grabens) by microorganisms (fungi, aerobic bacteria, insects, etc.). Further decomposition by anaerobic bacteria extracts oxygen

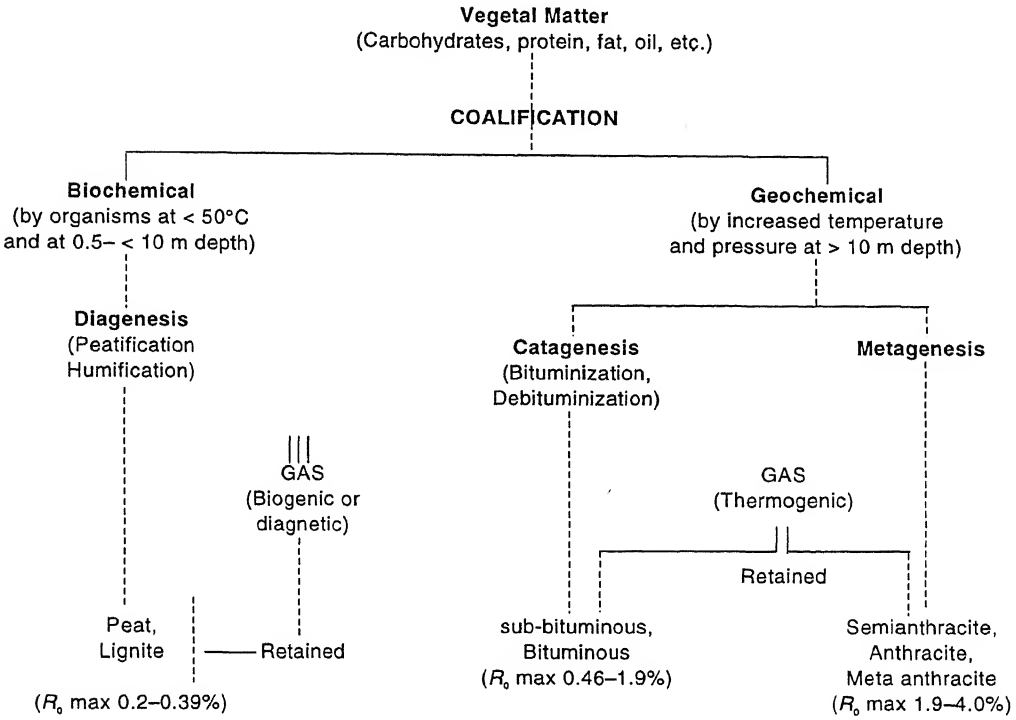

Figure 1. Flow-chart of methane gas generation during coalification.

from organic molecules of vegetal matter and results in high concentration of hydrogen. Part of this hydrogen is released as methane or 'marsh' gas and the rest is absorbed by humic colloids.

During subsequent geochemical stage of coalification, rising temperatures and pressures, due to subsidence of the basin/graben, either by growing thickness of overburden or by tectonic activities, generate hydrocarbons (hydrogen-rich constituents). Thermal cracking of the free lipid hydrocarbon fraction and/or cracking of the kerogen fraction of coal generates methane gas. Thus, the generation of coal bed methane during coal formation occurs in two ways:

(i) by metabolic activities of biological agencies (biological process), and

(ii) by thermal cracking of hydrogen-rich substances (thermogenic process).

Methane generated at shallow depths (<10 m) and lower-rank stage (sub-bituminous) by the first process (active up to 50°–80°C) is termed 'biogenic' or 'diagenetic methane' (Figure 1). Methane generated during this process is about 10% of the total methane generated by subsequent steps of coalification (catagenetic: > 80°–150°C, R_0 max > 0.50–2.0% and metagenetic: > 150°–200°C, R_0 max > 2.0–4.0%). Though most of the gas generated during early stages of coalification generally escapes into the atmosphere through the exposed peat or due to low hydrostatic pressure, some amount can accumulate under certain specific geologic conditions like rapid subsidence and burial, and thus may get trapped in shallow reservoirs.

Gas produced at greater depths and higher rank stages of the second process, the thermogenic methane, constitutes bulk of the coal bed methane. The gas generation, by this process, begins at vitrinite reflectance (R_0 max) values of 0.70–0.80%, peaks near the boundary between medium-volatile bituminous and low-volatile bituminous coal stages [R_0 max 1.1–1.4% (maximum at 1.2%), temperature 100°–150°C (ref. 3), Figure 2 *a*], and declines further with the rise in temperature and reflectance values[4,5]. Thus, it could reasonably be presumed that the prospect of generation of coal bed methane is more in the regions of high palaeogeothermal gradient as well as in the vicinity of intrusive bodies.

Although, methane is the major gas component of coal gases; water, carbon dioxide, wet gases and liquid hydrocarbons are also released during coalification. Total amount of methane generated during the coal formation (between R_0 max 0.5–2.0%) approximately ranges between 2000 and >5000 Scf/ton (ref. 6) (Figure 2 *b*). However, part of methane generated is retained in coal beds/seams and is termed 'coal bed methane' (CBM);

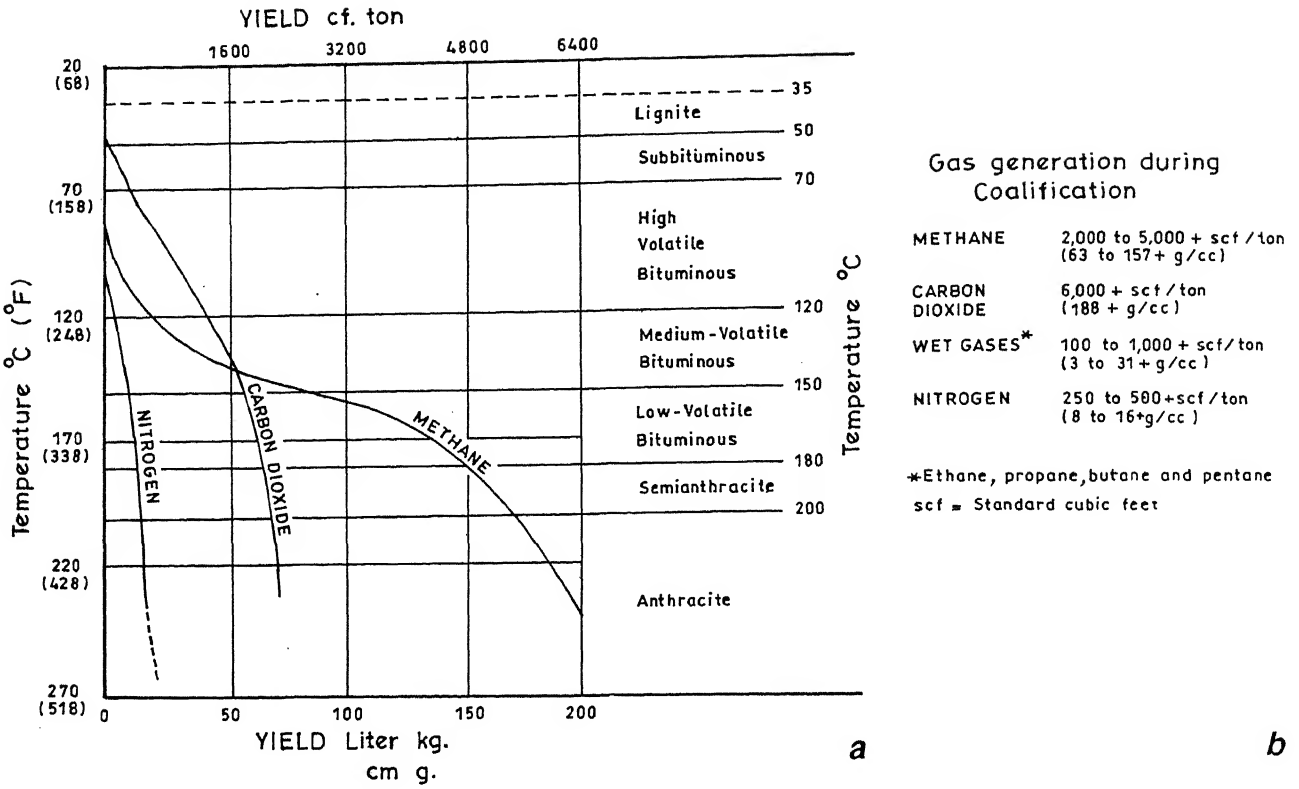

Figure 2. *a*, Relationship between gas generation and coal rank (source Manjrekar[3]); *b*, Gas volumes generated during coalification up to vitrinite reflectance values of 2.0% (after Scott[6]).

and the excess above the retention capacity of the coal bed, tends to migrate to the surrounding reservoir rocks (e.g. sandstones). Retention of methane in the coal beds is[7]:

- as 'adsorbed' molecules on internal surfaces or 'absorbed' within the molecular structure of the coal
- as gas molecules held within the matrix porosity (macro- and micro-porosity)
- as free gas within the fracture network
- as gas dissolved in groundwater within the coal bed.

Since methane is generated during coal formation processes, all coals invariably contain methane. However, the gas content of the coal normally increases with (i) rank of the coal, (ii) depth of burial of the coal seams, provided the roof and overburden are impervious to methane and (iii) the thickness of the coal seams.

Content of coal bed methane is assessed by several factors, amongst which the rank of the coal is the most important. According to Tang *et al.*[4] economically important quantities of methane (> 300 Scf/ton) are generated by thermogenic process, since large quantities of gas production are impossible until a certain threshold of thermal maturation is attained. This requirement is met in the high-volatile A bituminous rank at R_0 max between 0.8 and 1.0%. Investigations, world over, have shown that high rank coals buried at great depths (> 300–1200 m or more) are suitable for coal bed methane exploration, provided certain other geological and inherited coal seam characteristics are favourable as well.

Generally, gas is more concentrated in geologically active areas, such as folded and faulted regions as well as the surrounding areas of the faults. The well-developed cracks and fractures in the coal seams owing to tectonic disturbances, provide permeability to coal seams. The permeability of the seam is also related to the cleat system present in coals. The sealing capability and thickness of the seam roof and floor rocks play a significant role in methane accumulation. The fluvial basins, having higher rate of subsidence accompanied by thermal events and moderate tectonism, are the prospective sites for the exploration of coal bed methane.

The methane-generating capacity of coal is, however, related to the coal macerals. Whereas macerals of the vitrinite and liptinite (or exinite) groups are the greatest contributors of methane gas, the macerals of the iner-

tinite group have relatively little hydrocarbon generating potential, though they have the greatest capacity for storage of methane. Of the exinite groups, the liptinite macerals have the highest gas generating potential. Besides the cleats and other fracture systems, the mesopore structure of certain macerals (including structured inertinite) significantly enhance the permeability of methane within the coal seams. Therefore, vitrinite-rich coals of higher rank are reasonably the most important sources of coal bed methane for they have more micro-porosity (that is much higher absorbing capacity) than the other two maceral groups. Ash content of the coal also has an influence on the coal bed methane content: lower the ash content; higher is the gas content of the coal seam.

Methane gas sorbed on coal particles can be liberated by desorption of coal seams. The gas pressure in coal seams is released either by dewatering the coal seams or by drilling borewells which facilitate the flow of gas through fractures. As stated earlier, the amount of methane produced depends on desorption capacity of coals, which varies from coal to coal depending on its physical and chemical properties, especially the type of coal (*sensu* maceral composition). The amount liberated however, may be enhanced by using stimulation techniques as have been in practised in USA. Existing techniques of methane production being expensive, many companies are engaged in developing appropriate technologies for cost-effective production of this gas.

Status of coal bed methane in India

The prospect for coal bed methane is mainly related to the coal resources of the country. India has huge Gondwana (mainly Permian, 99.5%) and Tertiary (Eocene and Oligocene) coal deposits distributed in several basins located in peninsular and extra-peninsular regions. About 204 billion tons of coal reserves have been established and approximately 200 million tons or so are likely to be added in the near future by further explorations. The main Gondwana coal basins are rifted intracratonic grabens having thick sequence of coal seams, and hold considerable prospects for coal bed methane. The major part of Indian Gondwana coals (mostly up to 300 m depth) is of low rank, far below the threshold value of thermogenic methane generation. However, high rank coals, amenable for generation of coal bed methane, mostly occur in untapped deeper parts of basins covered by younger sediments.

Tertiary coals of India, occurring mainly in lagoonal to deltaic sediments, are better in quality compared to Gondwana coals, though the seams are thinner. On the basis of composition and rank of coal, Tertiary coals appear to be moderately rich in coal bed methane. The estimated coal bed methane resource of Gondwana coals appears to be between 1 and 1.5 Tcm and the Tertiary coals of about 4.3 Bcm (ref. 7).

In 1990, efforts to exploit coal bed methane were initiated by Essar Oil (a private oil company) under the advice of American experts. The methane emission and desorption studies on Gondwana coal samples from Jharia Coalfield (Bihar) were carried out by Central Mine Planning and Design Institute Limited (Ranchi) and Central Mining Research Institute (Dhanbad). The content of gas and gas emission rate from these samples were found to be 1.8–2.3 m^3/1000 m^2 of surface and 12.7–17.3 m^3/min, respectively[8]. The studies carried out by Bharat Coking Coal Limited in the same area with the help of French experts indicated 0.68–1.45 m^3/min gas emission rate.

In 1992, assessment of coal bed methane potential for Damodar Valley coals[7] was initiated by Oil and Natural Gas Commission. Till date, it has collected significant data related to coal bed methane exploration from drills in Raniganj basin. Recently, ONGC for the first time in country has succeeded in flowing the gas from seam no. XIV in Parbatpur block of Jharia basin[9].

Besides, Geological Survey of India and Reliance Industries Limited have also undertaken investigations on the prospects of occurrence of coal bed methane in different Gondwana and Tertiary coalfields of India. These investigations led to the delineation of potential areas in Damodar (Raniganj, Jharia, Bokaro, Giridih), Son (Sohagpur) and Pench-Kanhan-Tawa Valley (areas lying on the dipside of the Kanhan Valley) coalfields where a total gas-in-place reserve of 13.34 Tcf has been predicted[10]. In addition, gas content of 250 Scf/ton in an area of 900 km^2 has been recorded from Early Paleocene coal beds (50 m thick) of Cauvery Basin by Essar Oil Company[11].

Methane hydrate

Presence of methane gas in coal seams has been known since the very beginning of the coal researches. However, its presence in ocean bottom sediments as 'hydrate' is a relatively recent discovery. Mysterious disappearance of ships, sudden plane crashes and many strange events on land have unfolded the mystery of 'Bermuda Triangle' in Atlantic Ocean (*The Times of India*, Lucknow edition, 11 January 1998, published through *The Sunday Times*, London). The Bermuda Triangle, earlier considered as the area of supernatural power, is now known as a powerful source of energy with huge accumulation of methane gas in the form of hydrate. According to United States Geological Survey, two very small areas of north and south Carolina coast lying in a part of Bermuda Triangle contain gas equivalent to about 70 times the annual gas consumption of USA. The encouraging findings point towards high po-

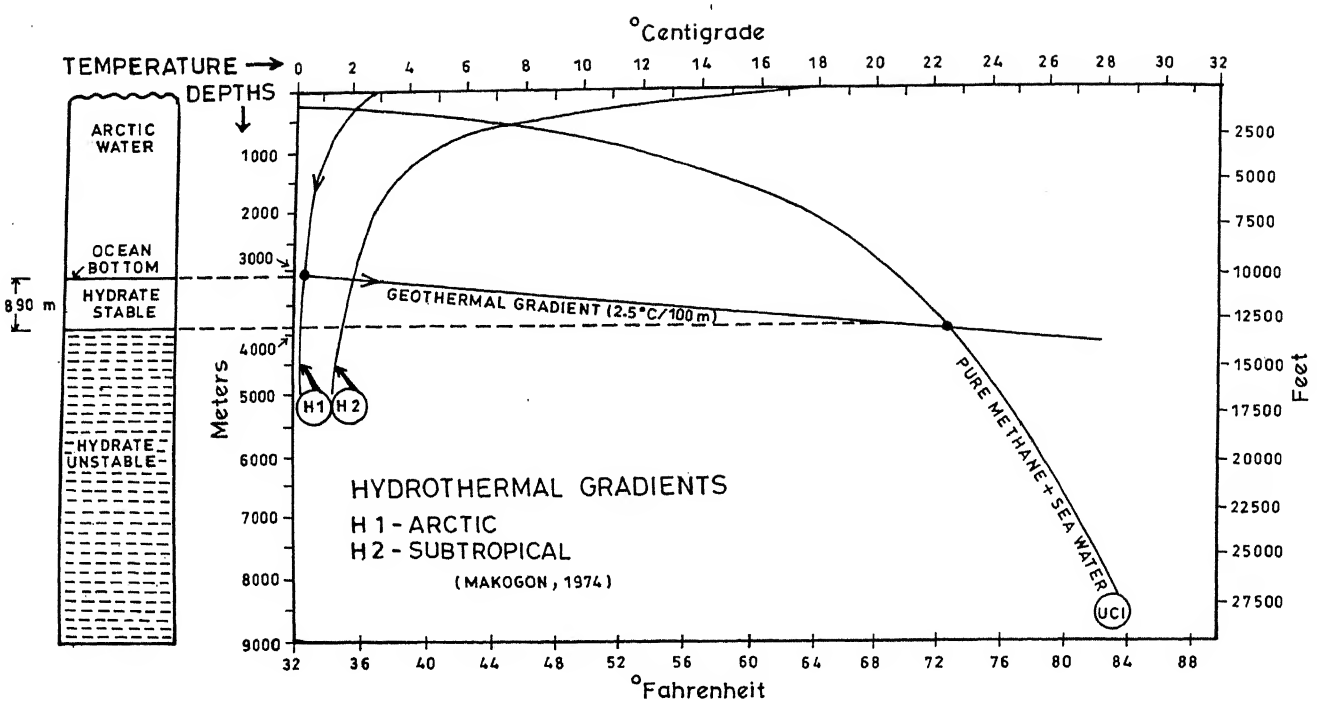

Figure 4. Temperature–depth (pressure) phase diagram of gas hydrate stability (after Macleod[25]).

ozone holes and global warming; traps atmospheric heat approximately 24-times more than carbon dioxide.

Thus, conversion of the most hazardous gas methane into a commercial energy resource, besides solving the world's energy problem for the coming century, will be advantageous in many other ways as mentioned below. The hazards involved in the exploitation of the hydrate deposits require development of suitable methods. Consumption of methane, which has the highest heat retaining capacity and the lowest atmospheric life-time span (about 10 years), will mitigate the global warming at a much faster rate. Consequently, methane hydrate – our future powerhouse – adversely affected by global warming, will be saved.

Emergence of methane gas as an additional energy resource warrants rational utilization and preservation of coal deposits, which are both the 'producer' and 'reservoir' of this gas and besides, burning can also be utilized for the exploration of methane gas. These vast energy reserves can be saved by selective utilization of coal.

Many leading nations have already launched projects to extract methane gas from coal beds and hydrates. However, continuous release of methane gas into the atmosphere during degasification of coal mines, by chlorofluoro carbon (cfc) compounds, and by hydrates adversely affects the global climate as well as human beings. Furthermore, the highly explosive nature of this gas, resulting in serious fires and accidents on land, water and air, has raised a few concerns on its utilization as a source of energy. At present, India too has all the scientific and engineering skills required for the production of methane from coal bed and seabeds. There are substantial unconventional energy (CBM and CH_4-hydrate) resources, but their exploration and economic exploitation from inaccessible areas are great challenges.

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Received 16 November 1998; revised accepted 22 March 1999

REVIEW ARTICLE

Lipoprotein (a): Biology and role in atherosclerotic vascular diseases

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Lipoprotein (a) (Lp(a)) is a genetically determined lipoprotein molecule. Its constituent, low density lipoprotein cholesterol participates in the process of atherosclerosis, and its prothrombotic tendency is due to its homology to plasminogen. In normal subjects plasma levels of Lp(a) are controlled by the apolipoprotein(a) (apo(a)) gene locus. The polymorphism of apo(a) is determined by more than 34 alleles, plasma levels of Lp(a) being inversely correlated to its isoforms. Plasma levels in healthy subjects are highly variable, and also depend on the ethnic group studied. Indians, both immigrant and native, display high plasma level of Lp(a). Many studies record them to have higher levels than most of the other ethnic groups. It is now established as a powerful and independent risk factor for macrovascular diseases due to atherosclerosis, including coronary artery disease (CAD), stroke and peripheral artery diseases. High levels of Lp(a) (> 30 mg/dl) appear to increase the risk of premature CAD. The risk is increased several-fold in the presence of high levels of other lipid and non-lipid risk factors. Lp(a) plasma concentrations are abnormal in other diseases, nephrotic syndrome and chronic renal failure and possibly in insulin-dependent diabetes mellitus as well. Unfortunately, this lipoprotein is not readily amenable to therapeutic intervention. Predictive value of this lipoprotein, and its observed high plasma levels makes it an important research, investigative, and prognostic tool, particularly for the Asian Indian population.

FACTOR(S) presumed to be responsible for increased propensity to atherosclerosis in Indians are abdominal obesity, smoking, glucose intolerance, resistance to the insulin-mediated glucose uptake, and a peculiar dyslipidemic profile[1,2]. The dyslipidemia seen in Indians include high triglycerides (TG), low high density lipoprotein cholesterol (HDL-c), and increased lipoprotein(a) (Lp(a)). A new dimension has been provided by the emerging role of Lp(a) and its importance in atherosclerosis-prone Indians. The following discussion shall outline in detail the genetics, biochemistry, metabolism, and clinical relevance of this lipoprotein.

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Lp(a): Biology and genetics

Lp(a) is a cholesteryl-ester-rich lipoprotein of unknown function formed by the covalent disulfide linkage of apolipoprotein (a) (apo(a)) to apolipoprotein B (apo B) of low density lipoprotein cholesterol (LDL-c). Recent studies of Lp(a) using scanning force microscopy showed for the first time a belt-like structure of apo(a) with both ends attached to a spherical LDL-c. The two ends of apo(a) were bound to the LDL-c sphere at two distant sites[3].

Apo(a) is a glycoprotein with approximately 29% (w/w) carbohydrates[4] and resembles plasminogen, the plasma zymogen for plasmin[5]. The apo(a) gene contains between 12 and 51 copies of a DNA sequence encoding a tandemly repeated cysteine-rich motif called kringle IV(K-IV), which is found in one copy in the plasminogen gene[6]. Apo(a) exists in multiple genetically determined isoforms with molecular weights ranging from approximately 350 to 1000 kDa. The number of K-IV repeats is inversely correlated with the plasma Lp(a) concentration[7,8].

The apo(a) and plasminogen cDNAs have been cloned[9–11] and the two genes are closely linked on chromosome 6(q26-27) (refs 12–16). Analysis of the apo(a) cDNA revealed a characteristic structure consisting of numerous K-IV repeats, one K-V, and a protease domain. These are homologous to corresponding structures in plasminogen mimicking its functional properties as well. This could partially explain the thrombogenic properties of Lp(a)[2].

Apo(a) occupies a unique evolutionary niche, mainly restricted to old-world monkeys, apes, humans and hedgehogs[17]. Primate and hedgehog apo(a) genes probably evolved independently from different domains of the plasminogen gene and are conspicuous examples of convergent evolution. Genetic size polymorphism of apo(a) has been demonstrated at the protein[7], mRNA[18] and DNA levels[19,20]. This polymorphism of apo(a) was originally identified by Utermann and his co-workers by SDS-polyacrylamide gel electrophoresis. The isoforms were designated as F, B, or S based on the pattern of mobility that is either faster, equal to or slower than apo B-100. Sophisticated separation techniques such as SDS-agarose gel electrophoresis have allowed identification of more than 34 apo(a) size isoforms[21]. Pulsed-field gel electrophoresis of genomic DNA digested with appropriate restriction enzymes from subjects with different apo(a) protein isoforms confirm that the size variation in the apo(a) protein is a result of a variable number of K-IV repeats at the apo(a) gene locus. There exists a perfect correlation between the size of apo(a) DNA fragments, size of protein isoforms and apo(a) mRNA size. However, some apo(a) alleles are not expressed at the protein level, and the expression of individual alleles in terms of plasma apo(a) concentration varies widely. Information on both the size and the degree of expression of an allele is obtained by performing apo(a) DNA and protein typing. The apo(a) gene locus is thus best described as a transcribed and translated VNTR locus that determines the extensive variation in size and concentration of the apo(a) protein.

Metabolism of Lp(a)

The largely varying plasma concentration of Lp(a) is randomly distributed in the population and correlates inversely with the molecular mass of apo(a). This protein is shown to have an unusual secretory pathway, mostly derived from studies of the intracellular metabolism of apo(a) in transfected human hepatoma cells and in primary baboon hepatocytes[22]. It is known that apo(a) is synthesised in the liver. *In vivo* turnover studies have revealed that variations in the plasma levels of Lp(a) are due to its synthesis rather than the degradation. An immature precursor form of apo(a) is retained in the endoplasmic reticulum for a prolonged time due to complex folding and processing. Since the retention time correlates positively with the apo(a) isoform size, this intracellular mechanism could explain the inverse correlation between the isoform size and the plasma concentrations. Another unusual feature of the biogenesis of Lp(a) is that the mature Lp(a) complex is formed only following separate secretion of apo(a) and LDL-like particles. Upon secretion from hepatocytes, apo(a) is assembled with plasma LDL-c to form Lp(a)[23]. This process requires docking and formation of a single disulfide bond between apo B-100 in LDL-c and K-IV in apo(a)[24–26]. The metabolism of Lp(a) is independent of other lipoproteins[27]. The major source of circulating plasma Lp(a) is the human liver. Serum Lp(a) concentration is determined by the rate of Lp(a) production and correlates directly with hepatic mRNA abundance[28,29]. Studies in primary cultures of baboon hepatocytes showed that the majority of apo(a) is secreted by liver cells into the medium in its free form[30]. Wilkinson *et al.*[31] showed that apo(a) in the human liver is not associated with apo B-100 and occurs extracellularly after secretion. The assembly of apo(a) and LDL-c, which is determinant for plasma Lp(a) levels, takes place extracellularly and requires specific structural motifs in apo(a) and apo B. Frank and Kostner[32] studied structural features of apo(a) necessary for high efficient assembly. According to their observations, K-IV T6 and T7 recombinant constructs were responsible for the high-yield assembly and K-IV T5 had an amplifying effect.

There are conflicting observations regarding the catabolic pathways of Lp(a). Because of its resemblance to LDL-c, it was initially postulated that Lp(a) degradation

was mediated by the LDL receptor (LDL-R). The apo B-100 in the Lp(a) may interact with the LDL-R[33–35]. However, the role of LDL-R in the removal of Lp(a) from the plasma is not defined despite a number of *in vitro* and *in vivo* studies. Lp(a)/apo(a) receptors on macrophages, receptor-related protein, and the asialoglycoprotein receptor have been implicated[36]. Lp(a) binds to LDL-R and is removed more rapidly from the plasma of transgenic mice having over-expressed receptors[37]. However, catabolism of ^{125}I was not significantly reduced in patients with defective LDL-R function[38], suggesting that the LDL-R is not directly responsible for Lp(a) clearance *in vivo*. Further, recent studies suggest that mouse embryo fibroblasts do not take up Lp(a) via the LDL-R[35]. Lp(a) turnover studies have shown that approximately 70% of the apo(a) component of Lp(a) may be released in the circulation, and the rest is degraded via the LDL-R[39].

Huby *et al.*[40] have demonstrated the presence of two distinct structural domains in apo(a) linked by a flexible and accessible region located between K-IV-IV and IV-V. They isolated the Lp(a) particle following removal of the N-terminal domain by proteolytic cleavage; the residual particle (containing the C-terminal domain spanning the region from K-IV-V to the protease domain), is linked to apo B-100 by disulfide linkage, termed 'mini Lp(a)'. The observation that mini-Lp(a) exhibits the same binding affinity to fibrin as the corresponding Lp(a) suggests that the kringles responsible for fibrin binding are restricted to K-IV-V to K-IV-X. This observation is supported by failure of the N-terminal domain to bind to fibrin. N-terminal fragments of apo(a) have been detected in the urine of normal subjects, thereby indicating that part of the catabolism of Lp(a), which is largely indeterminate, could occur via the renal route[40]. This possibility is supported by the observation that the excretion of apo(a) fragments was lesser in patients with reduced renal function[41]. Collagenase digestion of Lp(a) released apo(a) fragments of similar size to those found in urine, producing a particle that could then bind to LDL-R[42].

Lp(a) estimation

Laboratory estimation is performed by radioimmunoassay, immunoelectrophoresis and using monoclonal antibodies. Antibodies are raised against either Lp(a) or apo(a) in the intact Lp(a) molecule since disassociation of apo(a) from apo B-100 decreases its immuno reactivity. If antibodies are raised against K-IV repeats, it would lead to heterogeneity due to variation in their numbers[43]. A recent report suggests that patients suffering from cornary artery disease (CAD) excrete significantly higher amounts of apo(a) into the urine than controls and that urinary apo(a) is a valuable predictor of CAD. Using urinary apo(a) as a marker for CAD has the advantage of easier sampling compared to plasma samples[44].

Apo(a) size polymorphism, Lp(a) concentration and ethnic variation

Apo(a) protein, mRNA and DNA size polymorphism studies demonstrate that the number of K-IV repeats in the gene and the resulting size of the protein are inversely correlated with Lp(a) levels in the plasma in all populations studied so far[7,8,19,20,45]. Mean Lp(a) concentrations and apo(a) isoform frequencies vary significantly between populations. The plasma concentration of Lp(a) has a skewed distribution that varies over a 1000-fold range in white populations, with most individuals having low plasma Lp(a). In whites, studies in sibpairs indicate that over 90% of the individual variation of the apo(a) gene may be responsible for it, however, in other ethnic groups the contributions of this locus may be smaller[45]. A pentanucleotide sequence repeat polymorphism (TTTTA) at position-1373 before the translation initiation codon of the apo(a) gene may be one of the factors associated with variable plasma Lp(a) concentration[46]. This polymorphism could account for about 10–14% of the inter-individual variations of Lp(a) levels in Caucasians[47,48].

Tibetans, Japanese and Koreans have Lp(a) levels similar to the Caucasians. Higher Lp(a) levels are observed in blacks. Close correlation between CAD and Lp(a) levels has been observed in Welsh, Germans, Swedish, Finnish, Icelanders, Austrians, Australians, Chinese, and Japanese[49–51]. Higher levels of Lp(a) compared to other ethnic groups have been recorded in Asian Indians in US[52], UK[53], and Singapore[45]. Further evidence has been provided by another study where mean levels of Lp(a) were nearly double in sons of Asian Indians with CAD compared to similar-aged sons of white parents[54]

Other factors affecting plasma levels of Lp(a)

Lp(a) levels, unlike LDL-c and apo B levels, do not vary with the age of the subject. Lp(a) is fully expressed in the first year of life. Hence, its tracking is more useful than other lipids[51]. Various endogenous and exogenous factors affect Lp(a) levels in humans (Table 1). These include hormones (growth hormone, estrogens), hypothyroidism, and alcohol consumption. Several renal diseases alter Lp(a) levels including renal failure and nephrotic syndrome. Successful renal transplantation leads rapidly to correction of Lp(a) concentrations, especially in patients treated with chronic ambulatory peritoneal dialysis with higher Lp(a) levels[55]. Further,

Table 1. Factors influencing serum lipoprotein(a) concentrations

Physiological factors
Age, ethnic groups, menopause, high saturated fat diet
Chemical compounds and drugs
Estrogen, progesterone, danazol, growth hormone, neomycin, niacin, alcohol, cyclosporin
Diseases
Myocardial infarction, renal failure, nephrotic syndrome, familial hypercholesterolemia

Table 2. Lp(a) and atherosclerosis: Pathophysiological correlation

Contributes to uptake of LDL-c and formation of foam cells
Inhibits plasminogen activation and fibrinolysis leading to procoagulant tendency
Release of cytokines
Release of growth factors, smooth muscle cell proliferation
Increased expression of adhesion molecules
Endothelial dysfunction
Interacts with other risk factors e.g. homocysteine

diseases such as rheumatoid arthritis and familial hypercholesterolemia increase the Lp(a) levels[56]. On the other hand, sex of the subject, other lipoproteins, other coronary risk factors, environmental factors and anthropometric parameters have no significant effect on the Lp(a) level[50]. Lp(a) levels are not readily amenable to manipulation by dietary restriction. However, in one of the studies, substitution of a baseline diet of polyunsaturated fat with medium-chain saturated fat led to reduction in Lp(a) levels by 30% (ref. 57). Other recent studies indicate that Lp(a) level is lowered by saturated fat (e.g. palmitic acid) diet[58]. Moderate drinking of alcohol lowers plasma Lp(a) levels[59]. If the alcohol was withheld from moderate drinkers, this led to increased Lp(a) levels by 64% (ref. 60). This may be an additional mechanism by which moderate alcohol drinking may be beneficial for atherosclerosis.

Except niacin and hormone replacement therapy, no other lipid lowering agent lowers Lp(a) levels[61–65]. Other drugs such as cyclosporin, danazol, and stanazolol can increase the La(a) levels.

Pathogenic effects of Lp(a)

Atherogenesis

Lp(a) provides a carrier system for LDL-c and promotes cholesterol accumulation in cells[66] (Table 2). Oxidized LDL-c and Lp(a) accumulate in excessive amounts in macrophages ('foam cells') forming fatty streak. Intact Lp(a) deposition has been demonstrated in the arterial wall and venous grafts[67,68] and atherosclerotic plaques[69]. A study of the atherectomy specimens showed a correlation of plaque alpha-actin and Lp(a) indicating a role for Lp(a) in plaque growth as well[70]. Further, it stimulates smooth muscle cell proliferation[71], avidly binds to arterial proteoglycans[72], and fibronectins[73]. Plasma homocysteine increases affinity of Lp(a) for fibrin, thus increasing its atherogenic potential[74].

Oxidized Lp(a) is also implicated in the causation of endothelium dysfunction[75]. The endothelium-dependent vasoconstrictive response to L-NMMA was enhanced in subjects with relatively high Lp(a) plasma levels, suggesting an increased basal production and release of nitric oxide[76].

In induction of atherogenesis, recent evidence indicates that Lp(a) involves adhesion molecules. An endothelial cell-activating effect of Lp(a) is potent surface expression of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. This may be an important event in the initiation of atherogenic disease[77].

Thrombosis

Endothelial cells due to surface-connected fibrinolytic system are important for fibrinolysis. Lp(a), because of its plasminogen-like apo(a), interferes with fibrinolysis due to inhibition of plasminogen binding to its high affinity sites[78]. Lp(a), to some extent, regulates the synthesis of a major fibrinolytic protein, plasminogen activator inhibitor-1 (PAI-1)[79]. These prothrombotic events are now considered essential to the genesis of atherosclerosis.

Lp(a) and the risk for atherosclerotic macrovascular diseases

Association of Lp(a) and CAD was first observed in 1974. The accumulated data have established it as an important inherited risk factor for the macrovascular diseases including CAD, cerebrovascular and peripheral vascular diseases[80,81]. Child–parent association specifically looking for CHD in parents and lipid levels in the offspring in Bogalusa Heart Study indicates that Lp(a) is a marker of CAD in adulthood[82].

Several case-control studies have demonstrated an association of elevated Lp(a) plasma concentrations with premature coronary atherosclerosis and myocardial infarction[83]. Lp(a) is considered to be ten times more atherogenic than LDL-c[84–86]. Relative risk of CAD is increased three-fold if the levels of Lp(a) are more than 30 mg/dl (refs 87, 88). Serum Lp(a) levels have been shown to correlate well with the presence, extent, severity and score of atherosclerotic lesions on coronary angiography[89–91]. The Scandinavian Simvastatin Survival Study provides independent confirmation that a high Lp(a) lipoprotein level is a significant CAD risk factor[92]. In Quebec Cardiovascular Study, however, Lp(a) was not an independent risk factor for CAD but appeared to increase the risk associated with other lipid

risk factors[93]. The pathogenic association of Lp(a) and CAD has been further emphasized in a symposium on this topic held in Oslo in May 1997 (ref. 94). In a meta-analysis, 12 out of the 14 prospective studies showed Lp(a) concentration to be increased in subjects later developing CAD[95].

Recently, some of the studies have suggested that the size of apo(a) is also important. Amemiya and co-workers evaluated 94 Japanese patients with angiographically proven CHD and observed a negative association between Lp(a) levels and apo(a) protein sizes. The authors conclude that apo(a) protein sizes are a significant predictor, and the genotype homozygous for the 8 (TTTTA)-repeats a possible predictor of the degree of atherosclerosis in CAD[46]. In some studies apo(a) size was a better predictor of the disease and its severity[96,97]. Male patients undergoing coronary artery bypass had smaller apo(a) isoforms than the controls though their Lp(a) levels were normal[98]. Theoretically, a combination of small apo(a) size and high Lp(a) concentration should be particularly deleterious.

A few recent studies, however, suggest that Lp(a) levels may not be under such strict genetic control in patients with atherosclerotic vascular disease as has been demonstrated for a healthy population[89,99]. Several studies have explored the association of Lp(a) in centenarians. Almost a quarter of the centenarians had high plasma levels without showing any atherosclerosis[100]. In another study, such subjects had higher Lp(a) levels than the controls[101]. Paradoxically, therefore, some critical level of Lp(a) may be useful for increasing longevity.

Lp(a) has also been noted to be an independent risk factor for peripheral vascular disease[102,103]. Association of elevated Lp(a) level and stroke is controversial. In a study, Lp(a) was observed to be increased in about one-third of patients with acute cerebral ischemia, but did not correlate to stroke characteristics or prognosis[104]

Relationship between Lp(a) and lipid and non-lipid risk factors

Correlation coefficients of Lp(a) ranged from 0.16 to 0.17 for total cholesterol, LDL-c, HDL-c, serum triglycerides, apo A-I, apo A-II, apo B, and truncal fat[105]. Atherogenic risk appears to be increased when there is a cluster of lipid abnormalities. Effects of serum Lp(a) on atherogenesis are increased by high LDL-c and low HDL-c levels. Lp(a) levels more than 30 mg/dl by itself lend a 3-fold risk of CAD[106]. Men with LDL-c values of more than 317 mg/dl and Lp(a) values of more than 30 mg/dl have a 16-fold increased odds ratio of having CAD vs those having an LDL-c level of less than 130 mg/dl and Lp(a) level of less than 10 mg/dl (ref. 87). Hopkins *et al.*[107] studying early familial CAD have reported that the risk associated with elevated Lp(a) was observed in persons having plasma total cholesterol 6.72 mmol/l (260 mg/dl) or higher or with a total/HDL-c ratio of more than 5.8. Indeed when Lp(a) was over 40 mg/dl and plasma total/HDL-c more than 5.8, relative odds for CAD were 25 in multiple logistic regression analysis. Further, if two or more non-lipid risk factors such as hypertension, diabetes, cigarette smoking, high total homocysteine, or low serum bilirubin were also present, relative odds were 122. Risk for the development of CAD can be calculated using 'Lipid Tetrad Index' (product of total cholesterol, triglycerides and Lp(a) values divided by the HDL-c level)[50]. High levels of Lp(a) were found to increase the risk associated with hyperhomocysteinemia by a factor of nine, and a simultaneous elevation in both having an odds ratio of 31 for CAD[107]

Lp(a) and diabetes

In diabetes, conflicting reports are available regarding prognostic significance of Lp(a) levels. A few studies record that it may be elevated in insulin-dependent diabetes mellitus[108]. Particularly, patients with microalbuminuria and proliferative retinopathy show higher Lp(a) levels[109]. Similarly, Lp(a) has been correlated to CAD in diabetics in some studies[110–112], while other trials do not show any such correlation[113]. South Indian non-insulin-dependent diabetes mellitus (NIDDM) patients with high Lp(a) levels, however, show good correlation with CAD[114].

Lp(a) in Indians and CAD risk

In a study of Chinese, Malay, and Indian newborns in Singapore, high levels of cord blood Lp(a) in Indians reflected the adult differences in CAD rates[115]. Anand *et al.*[116] have computed data from three studies, CADI Study, study on Asian churchgoers in Chicago, and SHARE investigation in Canada. In two of the studies, the difference between Lp(a) levels in Asian subjects was higher than North American whites. Particularly, in the sample from the SHARE study, the mean Lp(a) concentration for South Asians was 34.1 mg/dl vs 17.3 mg/dl in white Canadians.

Studies performed in the native Indian population also record increased levels of Lp(a) in patients with atherosclerotic vascular diseases[117]. In another study, Lp(a) levels in 114 consecutive patients undergoing coronary angiography were compared with controls. CAD patients had higher levels of Lp(a). However, those who were alcohol drinkers showed significantly lower Lp(a) levels[118]. In another study done on North Indian patients, apo(a) phenotypic polymorphism and its effect on Lp(a) levels was studied on 130 angiographically proven CAD patients and 130 age and sex matched controls. It

Table 3. Quartile distribution of serum Lp(a) levels (mg/dl) in CAD patients and controls

Group	Mean ± SD	Quartile distribution 1–25	50	75–100	Skew
Patients (n = 130)	42 ± 24	0.79–16.0	31.1	62.9–160	1.21
Controls (n = 130)	27 ± 27	0.69–7.8	17.7	36.6-147	2.13

was observed that low molecular apo(a) isoforms associated with high Lp(a) levels in the general population are significantly over-represented in the CHD patients compared to controls (Table 3)[119]. This observation is in agreement with other studies on Caucasian populations[120,121]. In a recent study on South Indian non-insulin-dependant diabetes mellitus (NIDDM) patients, Lp(a), along with age and HDL-c levels were associated with CAD in NIDDM patients[114]. Since there is increasing prevalence of NIDDM in Indians, this observation has ominous portends in terms of total burden of CAD in Asian Indians.

Perspective

Lipoprotein(a) is now established as a genetically determined predictor of atherosclerotic vascular diseases, and in particular premature CAD. Determination of both Lp(a) and apo(a) isoforms makes cardiovascular risk assessment more comprehensive. Homology to plasminogen enables it to interfere with the fibrinolysis, thus providing additional pathway for atherosclerosis. High levels of this lipoprotein, particularly in Asian Indians, is a matter of clinical concern. Since it is not generally amenable to the lifestyle measures, other lipid and non-lipid risk factors must be modified to decrease the risk in those with high Lp(a) levels. It could be a useful tool for guiding management strategy in the individuals with: (i) family history of premature CAD; (ii) normal total cholesterol and evidence of macrovascular disease; (iii) isolated hypertriglyceridemia; and (iv) those belonging to high-risk ethnic group.

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ACKNOWLEDGEMENTS. This study was supported by research grants from Department of Biotechnology, Govt. of India and Indian Council of Medical Research, New Delhi.

Received 26 February 1999; accepted 17 March 1999

Nanoscale measurements for computing Young's modulus with atomic force microscope

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Atomic force microscope (AFM), developed at Central Scientific Instruments Organization, Chandigarh, has been configured for load–depth indentation measurements, wherein 'the reverse path effect' of AFM force curve associated with the use of piezo actuators has been overcome by measuring *in situ* actuator displacement independently by a laser Doppler displacement meter (LDDM) to enable correction of the force curves. The measurements of elastic moduli of highly oriented pyrolytic graphite, silicone elastomer, mica and gallium arsenide have been carried out.

THE force between the tip mounted on a cantilever beam and the sample surface as a function of the tip-surface separation can be computed with an atomic force microscope (AFM)[1]. The nanomechanical properties of the sample originating from the force associated with the tip–surface interaction have been studied by the AFM developed at Central Scientific Instruments Organization, Chandigarh[2].

Determining the mechanical properties of materials on nanometer scale, as desired in semiconductor industries and microsystems, requires special instruments having high lateral and depth resolution. This has been pursued by the nanomechanics and AFM communities[3]. In typical nanoindentation studies, the penetration of the indenter tip into the sample is measured as a function of applied load. AFM when used in an indentation mode, can measure the mechanical properties of surfaces with unprecedented force and lateral/penetration–depth resolution[4].

These AFM measurements are significantly affected both with regard to indentation curve shape and quantitative values of measurement by well-known effects of hysteresis and creep in lead zirconate titnate (PZT) piezo actuators used to control the positioning and motion of the mechanical components (cantilever, tip, etc.) of the AFM. An ideal behaviour is usually assumed for these actuators in which a reproducible displacement results for a given applied voltage, regardless of the magnitude of the voltage or the recent translation history of the actuator. Both these factors affect the response of the piezoelectric actuator, and the measured force curve is distorted when the tip is retracted from the sample. The unloading portion of the curve yields a higher applied load than the loading portion. This phenomenon called 'reverse path effect' is an instrumental artifact[5]. These adverse effects limit the use of AFM for quantitative measurements.

The problem could be overcome by removing the piezoelectric actuators[5] or by measuring displacement of the PZT actuator independently. In this study, the displacement has been measured optically by laser Doppler displacement meter (LDDM)[6] showing a measurement accuracy of one part per million. This electro-optical device detects the Doppler shift of a laser frequency caused by a moving target. The voltage–displacement plots of piezo actuator were obtained and corrections to the force curves were thus made.

The measurement of elastic moduli of highly oriented pyrolytic graphite (HOPG), silicone elastomer, mica and gallium arsenide (GaAs) have been carried out and presented in this paper.

Basically, a force microscope is comprised of a sensor (tip mounted on a cantilever which deflects due to force interaction between the sample and the tip) and a detector. The detector measures the cantilever position which is used to determine the force (F) on the tip using Hook's law i.e. $F = KZ$, where K is the stiffness of the cantilever and Z is the cantilever displacement.

Instrumental details of AFM configured for this work are shown in Figure 1, wherein 'A' shows the optical deflection system consisting of a laser diode, focussing optics, cantilever with its mount, and the position sensitive detector (PSD) to measure the deflection of the cantilever. AFM stage consisting of magnetic sample holder, differential micrometer operated coarse approach mechanism is shown in 'B', and 'C' shows the related signal processing hardware.

Force curves are the plots of the force on a cantilever tip as a function of the distance between the tip and the sample. To plot the force curve, a voltage signal in the form of a triangular wave is applied to the z piezo (for movement of the piezo in vertical direction). The sample whose properties are to be determined is mounted on the piezo. The signal causes the piezo to undergo a number of cycles of expansion and contraction and, thus, the sample approaches and withdraws from the tip repeatedly. When the sample approaches the tip, the cantilever remains undeflected until the tip and the sample are close enough to interact. Figure 2 shows a typical force curve.

Force curves for indentation studies, unlike AFM imaging, require exact measurement of cantilever and sample positions, as the accuracy in determination of these displacements would influence other measurements obtained from the force curves. Sample movement is through the piezo whose exact response to applied

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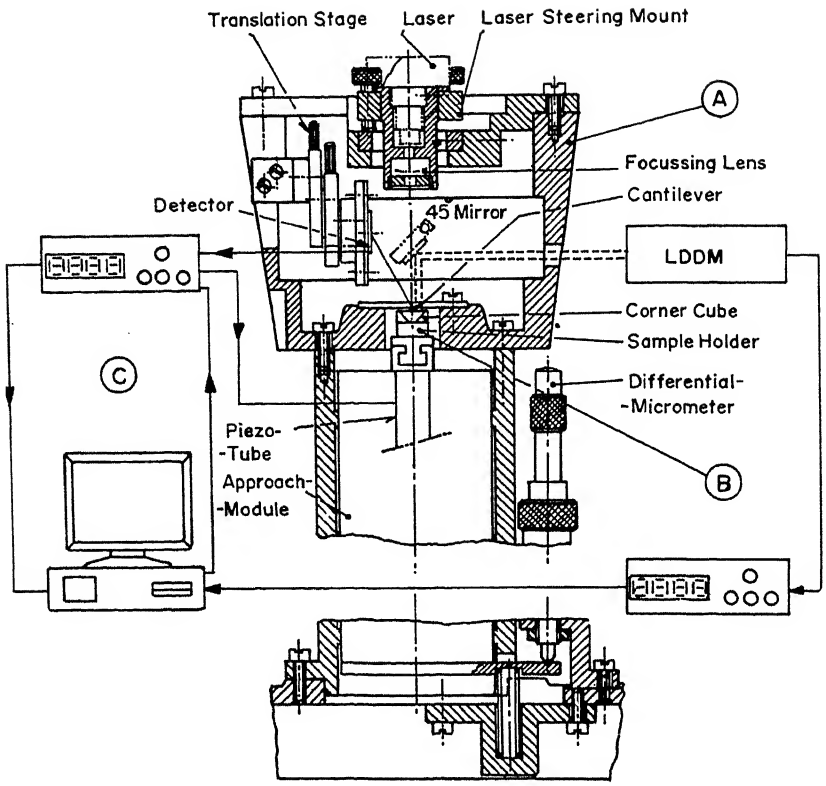

Figure 1. Instrumental details showing AFM integrated with LDDM.

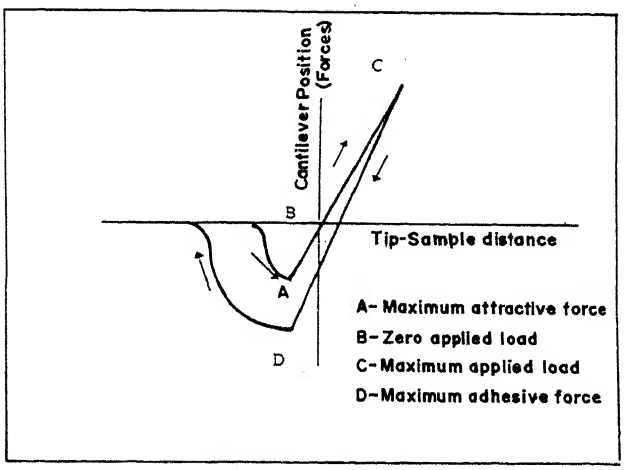

Figure 2. Typical AFM curve.

voltage is required for determining the properties of the sample.

The piezo actuator used in this study was a radially polarized PZT tube with an outer diameter of 12.7 mm, length of 68 mm, and wall thickness of 0.7 mm. The change in length of the tube actuator can be calculated[7] from the following equation:

$$\Delta L = (L/W)\, d_{31} \cdot V,$$

where L is the length of the piezo tube, W is the wall thickness, d_{31} is the piezoelectric strain coefficient and V is the applied voltage. Using the manufacturer's value of -173×10^{-12} m/V for d_{31}, the computed sensitivity is 16.6 nm/V, whereas the measured value from the force curve (Figure 3 *a*), taking linear behaviour is 24 nm/V. This is 47% higher than the calculated value. This is quite in order, as it has been observed by other authors[5] as well.

In situ measurements of displacement of this tube were carried out by placing a corner cube and introducing a mirror at 45° as shown Figure 1. Laser beam from the LDDM was targeted on the corner cube and the reflected beam was aligned to the phase detector in LDDM. AFM and LDDM were interfaced with the computer through their respective controllers.

The expansion and contraction of the piezo tube was controlled by the use of an appropriate computer software. Voltage in the form of triangular waveform was applied to the piezo with the help of DAC card DT-2821 (Data Translation Marlborn, MA). Doppler shift of the laser frequency caused by displacement of the piezo tube was detected by the phase detector, as the frequency of the reflected beam is proportional to the

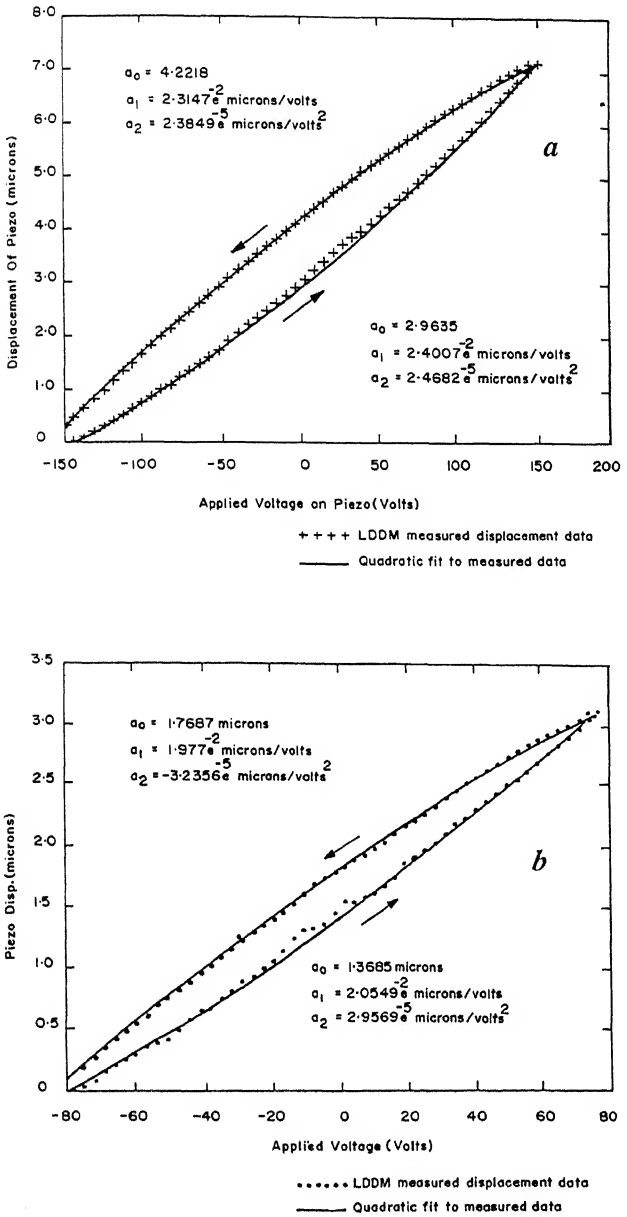

Figure 3 *a* and *b*. Actuator response as a function of applied voltage.

displacement. Digitized detector output was connected to the computer through RS-232-C port. Two sets of experiments were carried out. In the first set, the piezo tube was moved to its full range i.e. from –150 V to +150 V and back to –150 V. In the second set, voltage range of –75 V to +75 V was used. The waveform was applied through a DT card, whose voltage range was –10 V to +10 V. The voltage range was divided into 100 equal steps, the steps of 81 DAC units (0.4 V) for the full range and 40 DAC units (0.2 V) for the half range. Thus, the displacement of the actuator was studied over the specified voltage range which is required for matching sample loading through the same piezo expansion rate. Triangular waveform used for the movement of the piezo tube had a time period of 26.6 s. This is significant, as this time period matches with the pixel–pixel delay period, while acquiring the force curve data.

This data was used to evaluate a second-order polynomial, $D = a_0 + a_1V + a_2V^2$, wherein a_0, a_1, a_2 are constants, D is the displacement and V the applied voltage. Two separate polynomials were obtained from Figure 3 *a*, *b*, one for the expansion and the other for the contraction, which describe the response of the actuator accurately. Each polynomial was used separately while studying the loading–unloading of sample through expansion–contraction of the piezo actuator.

Determination of cantilever displacement (obtained with optical deflection technique)[8] requires accurate calibration of PSD. Detector voltage to distance relationship is determined by the movement of detector stage micrometer and recording the change in PSD output voltage. This calibration has been used to convert the measured detector response to distance. The detector response was measured to be 1.2 mV/μ, the length of the cantilever was 85 microns and the distance between the cantilever and detector was 32 mm.

Alternatively, the detector calibration can be done from the initial part of the loading curve, when the sample just comes in contact with the cantilever. At this point, the movement of the cantilever can be considered as the movement of the sample itself.

HOPG, silicone elastomer, mica and GaAs samples were taken for this study. HOPG and mica were prepared by exfoliation in air. GaAs was cleaned with hydrofloric acid. All measurements were performed in a glove box, containing dry nitrogen. The glove box was thermally insulated.

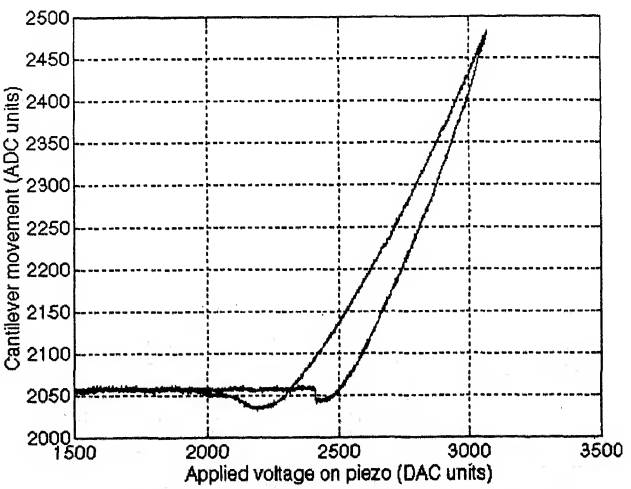

Figure 4. A typical plot of cantilever movement as a function of applied voltage on actuator.

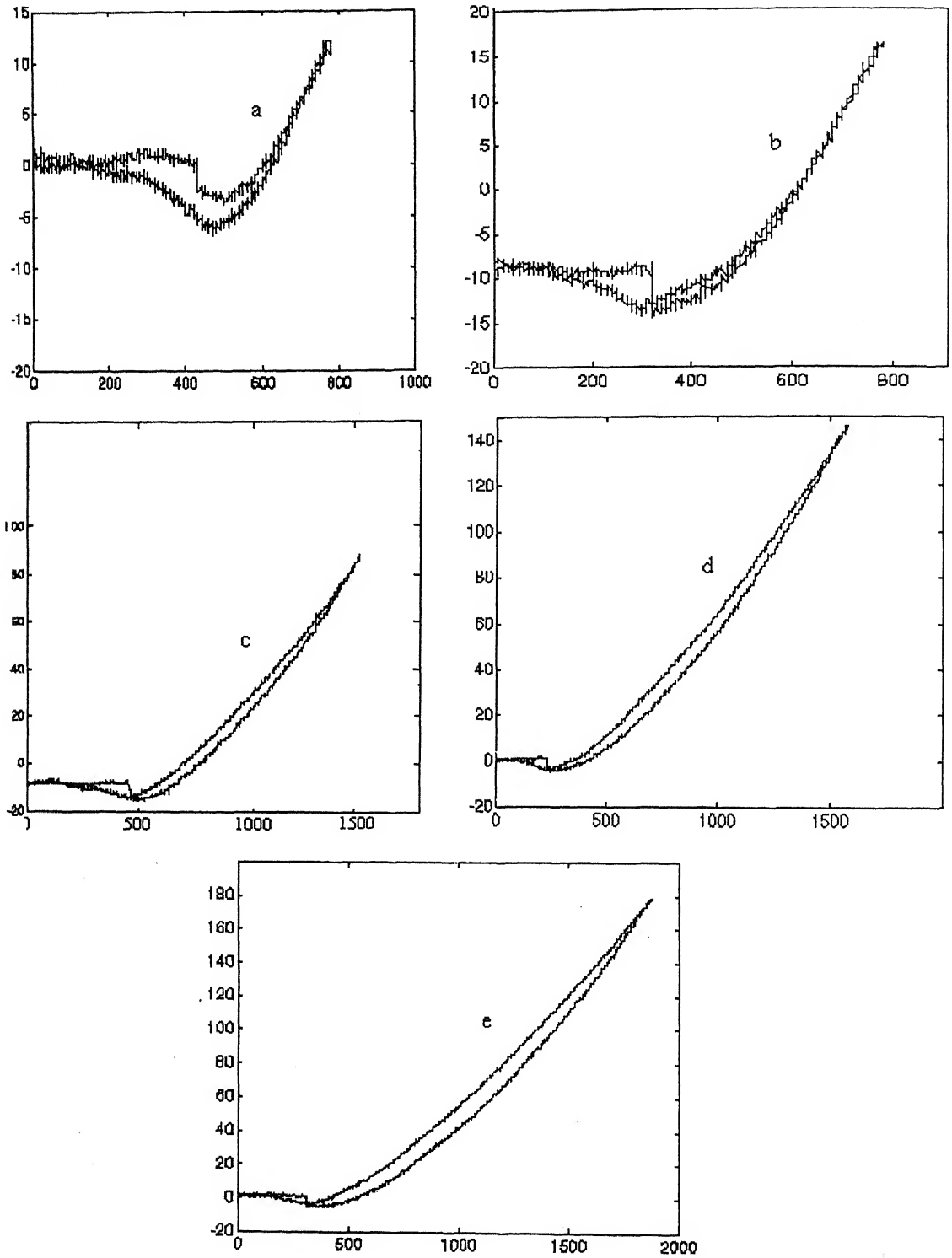

Figure 5. The corrected force curves for various loads, without reverse path effect for silicone elastomer.

The piezo tube carrying the sample was moved in z direction in ranges over which it was calibrated, as described earlier. The force curve data was obtained by giving a triangular wave voltage with minimum DAC step (12 bit resolution) to the sample actuator.

For measuring the cantilever movement, PSD output was taken through ADC channel with a gain of 8 and 4 (range of ± 1.25 V and ± 2.5 V, respectively). For every increment in the DAC step, the corresponding cantilever movement in the form of ADC data was stored and displayed (Figure 4). From the stored data, the force curves at different loads for silicone elastomer were plotted (Figure 5), using linear detector calibration and piezo calibration polynomials. Two different polynomials were used for loading and unloading of the sample. These curves are free from reverse path effect.

Nanoscale indentation hardness of hydrogenated carbon thin films have been studied by Web and Komvopoulos[9] using atomic force and point contact microscopy. AFM images obtained with silicon nitride tips of nominal radius less than 20 nm demonstrated that carbon films possess very similar topographies and root mean square values in the range of 0.7–1.1 nm. Nanoindentation and wear experiments performed with diamond tips of radius equal to about 20 nm revealed a significant enhancement of the hardness and wear resistance with necessary film thickness.

The capability of AFM to quantitatively measure the nanomechanical properties of metals via nanoindentation using lead magnesium niobate (PMN) crystals instead of PZT have been illustrated using three single crystal of chromium, molybdenum, and tungsten by Hues *et al.*[10].

The HFM is being used to study a variety of phenomena including adhesion, tribology, and the mechanical properties of surfaces on the nanometer scale. As a result, the technique is evolving from a qualitative imaging tool to a quantitative probe. There is a serious problem associated with severe hysteresis and creep of PZT actuators used in these instruments, which limits their performance in quantitative applications. We have solved this problem by measuring *in situ* PZT actuator displacement independently by LDDM to correct the force curves, as detailed earlier.

Indentation parameters, load and penetration depth, are determined from the force curves plotted earlier (Figure 5 *a–e*). Here, the cantilever tip acts as an indenter and penetrates the sample as it is loaded. The depth of penetration has been obtained by subtracting cantilever movement from the sample movement in the loading range of the force curve. Tip load deforms sample according to its elastic or plastic behaviour depending upon the mechanical properties.

The deformation of the tip while studying the samples e.g. mica, HOPG, GaAs and silicone elastomers is negligible since the reported hardness of silicon tip material is much higher than the samples. Further, the loads applied while studying the samples are low and result in stresses well within their elastic limits.

Figure 6 shows that silicone elastomer behaves almost ideally. Load increases linearly with penetration depth

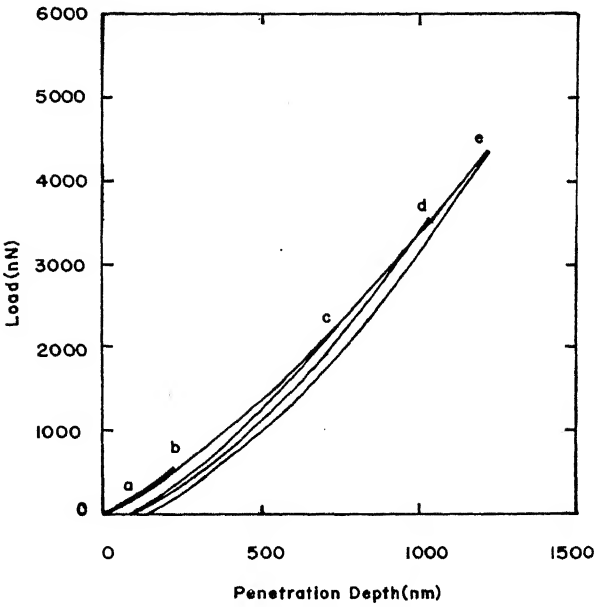

Figure 6. Penetration depth as a function of applied load for silicone elastomer.

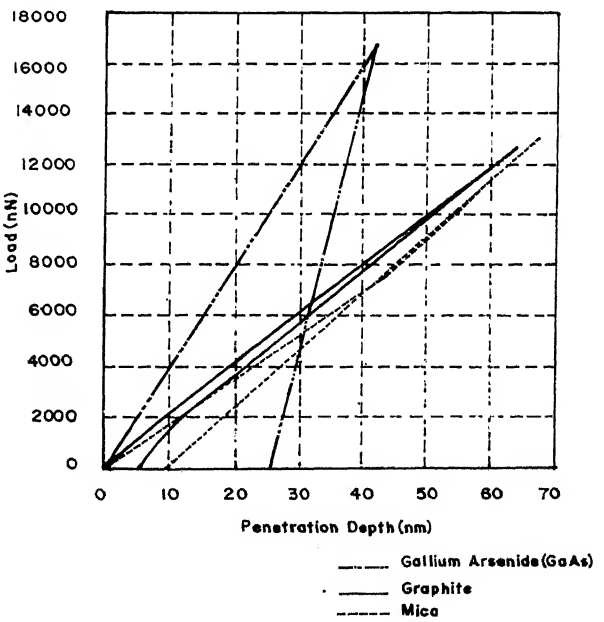

Figure 7. The load penetration depth plots for mica, HOPG and GaAs.

up to a load of 500 nN. For a load of 0.5 μN, the penetration depth is 220 nm. After this load, the sample deforms plastically leaving behind residual depth at zero load. This, however, increases with the applied load. For a maximum load of 4.4 μN, the penetration depth is 1200 nm. Each plot is based on an average of five readings of penetration depth at each point wherein the variation did not exceed 5%.

For mica, HOPG and GaAs, the load vs penetration depth results are shown in Figure 7.

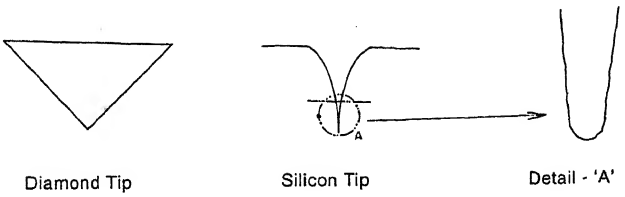

Figure 8. Geometry of silicon and diamond tips integrated with AFM cantilevers.

Table 1. Load (F), penetration depth (h) and computed and bulk volumes of the modulus of electricity (E) in the elastic range

Material	F	h	E (Computed)	E^{12} (Bulk)
Silicone elastomer	500	220	90.6 MPa	15–25 MPa
Mica	4500	40	5.6 GPa	15–20 GPa
Graphite (HOPG)	12000	59	10.1 GPa	5–25 GPa
GaAs	16150	45	17.8 GPa	70–90 GPa

The indentation plots show that the variation of load with penetration depth can be taken nearly linear for the loads in the elastic range of the materials. The relation between load (F) and penetration depth given by Snedden[11], is:

$$F = 2\,E\,r\,h/(1-\mu^2)$$ (for cylindrical flat ended indenter),

wherein E is Young's modulus of elasticity, r is radius of contact area (taken as tip radius), h is penetration depth, and μ is Poisson's ratio.

It is not necessary to have an accurate value of μ to get a reasonable value of E.

The silicon tips used in this study have much higher aspect ratio compared to diamond tips. The shape of silicon tip penetrating into the sample approximates a cylinder (Figure 8) and as such it is safe to use the above relationship.

Referring to Figure 6 (for silicone elastomer) and Figure 7 (for mica, HOPG and GaAs) for load vs penetration depth, the values of Young's moduli for these materials can be computed using Snedden's relationship.

In the elastic range, the values of load (F) and penetration depth (h), and the computed values of E have been listed in Table 1. In computing the values of E, a value of r equal to 9 nm is used. The values of μ used are 0.48 for silicone elastomer and 0.33 for the other materials.

AFM indentor tip may encounter layers of contamination and oxide layers which may have significant effect on mechanical properties; it may be a convolution of the properties of all the layers in contact with the tip. Actual dimensions of the tip and cantilever (which are taken from the commercial data) may be different from the values used. Hence the E (computed) values and E (bulk) reported in the literature differ widely.

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ACKNOWLEDGEMENTS. We thank all the members of STM/AFM group in CSIO, particularly Mr Narinder Singh, Mr Anil Sonkusare, Mr Pradeep Kumar, Ms Jasjit Kaur and Mr Vijay Mohal for experimental and technical support.

Received 16 November 1998; revised accepted 26 March 1999

Langmuir–Blodgett films of poly alkyl thiophenes: Preparation and characterization of multilayers

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The interfacial behaviour of poly alkyl thiophene monolayers formed at air/water interface have been studied using π-A isotherms. These formed stable condensed monolayers on water and could be transferred by the Langmuir–Blodgett (LB) technique. The UV visible spectra of the LB films showed that the polythiophenes form well-defined aggregates with their long axes nearly vertical to the layer plane. Optical microscopy in the Brewster angle set-up showed rigid striated structures in the case of hexyl-substituted polymer while the cyclohexyl derivative showed a less oriented monolayer. In the case of hexyl-substituted polymer (LB) films, the planar polythiophene main chains lie roughly edge-on parallel to the substrate while side chains are approximately orthogonal to the substrate.

IN the last two decades, increasing demand for new polymers has been evidenced in the area of functional materials, designed for specific applications often in the electronic and communication technologies system[1].

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π-stacking of π-conjugated polymers is a subject of recent interest and formation of π-stacked state is important for better performance[2] (e.g. large optical third-order non-linear susceptibility) of the conjugated polymer. Thiophene-based polymers are the subject of considerable interest due to the chemical versatility of the thiophene that lead to macromolecules with various structures and modulated physical and electronic properties[3]. Significant recent progress in the synthesis of thiophene-based polymers has resulted in enhanced processability and stability. The incorporation of relatively long and flexible side chains is a common technique for preparing soluble polymers having a stiff backbone[4]. The insolubility and infusibility of polythiophenes has been overcome by the controlled introduction of flexible side groups in the 3rd position[5,6]. The relationship between chemical structure, solid state organization and physical properties in this family of polymers thus seems highly significant[7–9]. In this regard, influence of molecular weight, regio regularity, polymorphism in octyl, decyl, and dodecyl derivatives of poly (3-alkyl thiophenes) have been well documented[10–12]. The use of LB film technique provides a well-defined arrangement of π-electron system in the layered structure and such organized monolayer assemblies containing quinquethiophene were reported with respect to their conductive properties[13,14]. However, the orientation of the functional molecules even in such arrangements is still controversial. A large number of studies using the LB film technique have been carried out with preformed polymers to achieve the necessary material property and strength[15–17]

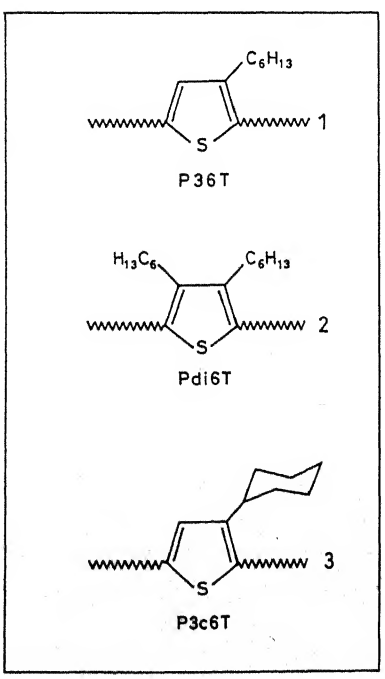

Figure 1. Structure of poly alkyl thiophene repeat units: 1, P3HT; 2, PdiHT; and 3, P3cHT.

In the present work, using three poly alkyl thiophenes (Figure 1), the molecular organization in LB films has been studied by forming monolayers at air/water interface. Poly alkyl thiophenes with different substitutents at the 3rd and 4th positions have been already tested for their mechanical stability using conventional thick films[18,19]. The mono layers formed from the poly alkyl thiophenes have been characterized by the surface pressure isotherms and the UV visible spectra of the films then transferred on to solid substrates by the LB technique have been analysed. The morphology of these layers has been studied by a Brewster angle microscope set-up.

Preparation of monomers and subsequent chemical polymerization have been done using reported procedures[19–21] and the three poly alkyl thiophenes were obtained. The purities were checked by spectroscopic and chemical analysis and were found to be 99% pure. The weight-average molecular weights of these substances found using GPC are between 90,000 and 1,50,000 and the polydispersity of P3cHT is (9.81) very high when compared to polymer 1 (P3HT) (3.87) and polymer 2 (PdiHT) (5.37).

The monolayers were formed by spreading chloroform solution on to distilled water or aqueous subphase containing 5×10^{-5} M $KHCO_3$ (pH 6.3). A NIMA trough (model 611) with a Wilhelmy-type balance was used for the measurement of the surface pressure-molecular area (π-A) isotherms. The LB films were built upon quartz substrates cleaned by the usual procedure (washing with H_2SO_4:H_2O_2 (7:3) and rinsing with double distilled water). The films were transferred at π = 15 mN/m and the transfer ratios were about 0.9. The UV visible spectra were recorded on a Shimadzu 160A spectrophotometer with a special set-up for the LB films. The optical micrographs were taken with a Brewster angle microscope set-up very similar to that of the Nanofilm Technologie, Gottingen, FRG.

Figure 2 shows the π-A isotherms for the compounds P3HT (1), PdiHT (2) and P3cHT (3) on $KHCO_3$ subphase at $T = 22$°C. The area/repeating unit at π = 15 mN/m for compound 1 is 11 A^2 while that for compounds 2 and 3 is around 16 and 18 A^2 respectively, suggesting that the planar poly thiophene main chains lie, roughly edge-on parallel to the water surface while the side chains are all perpendicular to the subphase. These monolayers are stable up to 18 mN/m above which they seem to collapse to form stacks of multimeric structures. Compared to compound 1 the other compounds seem to form condensed monolayers with smaller molecular areas. Both compounds 1 and 2 show a liquid expanded (up to 30 A^2) to liquid condensed state (15 A^2) without any other phase transition, while compound 3 shows a fairly large plateau of low compressibility (between 18 and 10 A^2). This may suggest that the polymer 3 with a cyclohexyl group[21,22] introduces in addition to a steric effect, a more hydrophobic character to the monomer units.

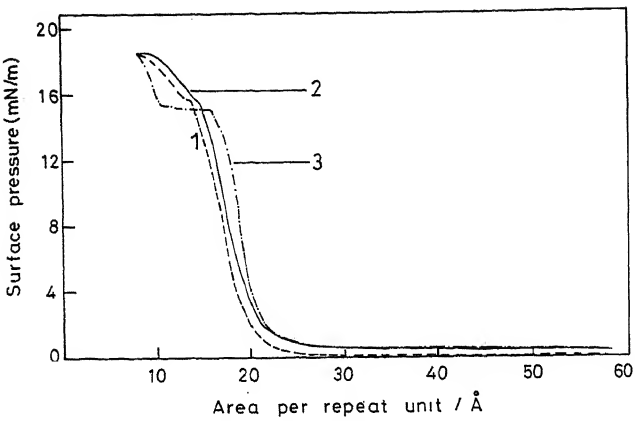

Figure 2. Surface pressure area isotherms for polymers at $T = 22°C$, 1, P3HT; 2, PdiHT; and 3, P3cHT.

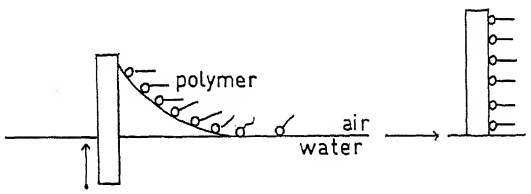

Figure 3. Schematic diagram of Y-type transfer of LB film.

These monolayers can be transferred on to solid supports at 15 mN/m to form a 'Y' type film[23] (Figure 3). However, the quality of polymer 2 in the LB film is not very homogeneous and therefore no spectra could be measured.

Figure 4 shows a representative spectra of compound P3cHT in solution and in LB films. The spectra does not show large differences for the three polymers. For the sake of brevity, they have not been shown here. It is seen that uniformly there is a shift to the red especially the 408 nm band in the solution which shifts to 430 nm. The band gap in the LB film was found to be 2.37 eV while the solution showed 2.56 eV. This long wavelength band shift may be assigned to the electronic transition along the long axis and indicate the formation of J aggregates. Further, the absorption bands at 275 nm in the solution exhibit a blue shift (268 nm) in the LB films. In a first approximation, from the dipole interactions between chromophores in the linear aggregates, it is expected that the absorption band due to the transition moment that lies flat exhibits the red shift while that standing vertically exhibits a blue shift[24]. These agree well with the λ_{max} values of pure poly (2,5 thiophene diyl) compounds with the values ranging between 480 and 500 nm and band gap being 2.0 eV. The shorter wavelengths with high band gaps in the present study may result from steric interactions between the substituents and the polymer backbone. These steric interactions lead to a non-planar conformation and consequently reduce the effective conjugation length. Though the solution spectra of all the compounds showed similar bands, the spectra in LB films seem to reflect strongly the effect of

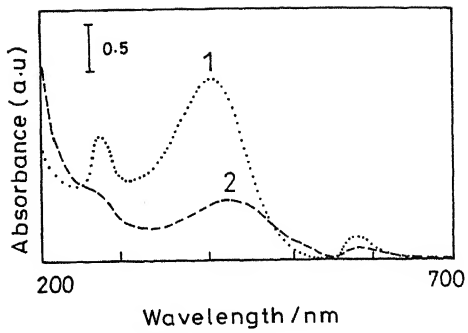

Figure 4. UV visible spectra of solution (1) and LB film (2) of P3cHT.

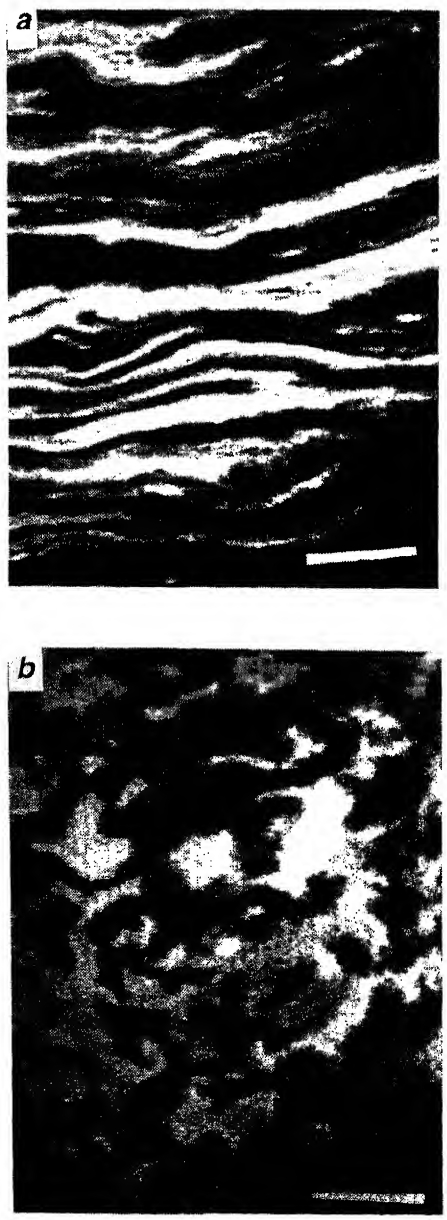

Figure 5. Optical micrograph of *a*, polymer 1 and *b*, polymer 3 at $\pi = 15$ mN/m (scale bar is 20 μm).

the different substituents. Such an effect should be related to inter-molecular effects (i.e. better packing)[25].

The optical micrographs of these LB films at Brewster angle set-up for compounds 1 and 3 are shown in Figure 5 *a* and *b*. Polymer 1 shows a fairly rigid structure with striations running parallel to the dipping direction. The micrographs also indicate that the rigidity leads to more disorganized domain formation with different orientations in the case of polymer 3. This compares well with the mechanical studies carried out on these polymers which showed that the anisotropy in polymer 3 would decrease as the substitutents increased in size.

This work shows that these pure alkyl-substituted thiophenes form stable monolayers at air/water interface and can be transferred to solid substrates by the LB technique. It is also seen that a possible change in orientation and packing of polymeric LB films can be obtained which can find applications in conductivity[22], nonlinear optical and electronic properties, especially in light emission[3], when the polymer was fabricated into light emitting diodes .

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Received 4 December 1998; revised accepted 16 March 1999

Expression of *npt*II marker and *gus* reporter genes and their inheritance in subsequent generations of transgenic *Brassica* developed through *Agrobacterium*-mediated gene transfer

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***Npt*II marker and *gus* reporter genes were introduced in *Brassica juncea* through *Agrobacterium*-mediated transformation showing a transformation frequency of 3–5% in the best responsive medium. Seventy-one per cent regeneration from hypocotyl explants was obtained on MS medium containing 0.01 mg/l 2,4-D and 2.0 mg/l BA supplemented with 20 µM $AgNO_3$, and 0.7% agarose as the gelling agent. The presence of transgenes (*npt*II and *gus*) in T_0, T_1 and T_2 generations was confirmed by dot blot analysis, using *npt*II gene as the probe, and by histochemical assay for the *gus* gene, respectively. Mendelian inheritance of the transgene (*npt*II) was observed in the T_1 and T_2 generations. Both the marker and reporter genes co-segregated in the T_1 and T_2 generations. The kanamycin-resistant plants in the T_1 progeny were either homozygous or heterozygous for the transgene.**

AMONG the various approaches for integrative transformation, *Agrobacterium*-mediated technique is most widely used. This method of transformation has been used previously by Mathews *et al.*[1], Barfield and Pua[2],

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and Pental *et al.*[3], in different species of *Brassica*. Most of the previous investigators have reported monogenic Mendelian segregation of the transferred genes in the first generation of transgenic plants.

In the present study, *Agrobacterium*-mediated transformation has been carried out in *Brassica juncea* cv. B-85 (B-85 is the most widely grown cultivar in West Bengal) using *npt*II gene (neomycin phosphotransferase II) as a selectable marker and *gus* (β-glucuronidase) as the reporter gene. In the construct used for transformation, the two genes are in the same cassette under independent CaMV 35S promoter. The *gus* expression was checked in different plant parts in T_0, and the segregation pattern of the transgenes was observed in T_1 and T_2 progenies of the original transformants.

The *Agrobacterium tumefaciens* strain LBA 4404 containing the binary vector, pPH26 (11.55 kb), was constructed from pPZP vector family containing *npt*II gene which confers kanamycin resistance, and *gus* gene which controls the expression of β-glucuronidase. Both the genes were under the control of a separate CaMV 35S promoter with opposite orientation and situated within left and right border sequences (Figure 1 *a*). The construct was a gift from P. Maliga, Waksman Institute, Rutgers University, USA. The bacteria were cultured overnight at 28°C Luria broth (LB: 10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl, pH 7.2) medium supplemented with 200 mM $MgCl_2$ and 50 mg/l kanamycin. The bacteria were then centrifuged at 4000 rpm, suspended in hormone-free MS medium[4] and the density was adjusted to $A_{600} = 0.1$.

The hypocotyl explants were precultured in regeneration medium (without $AgNO_3$) for one or two days before co-cultivation with *Agrobacterium*. Pooled lots of the explants were suspended in inoculum for 10 min, blotted dry on sterile blotting paper, and placed over regeneration medium without $AgNO_3$. After co-cultivation with *Agrobacterium* for 48 h, the explants were washed overnight in liquid, (hormone-free) MS medium, blotted dry, and placed over regeneration medium containing 250 mg/l cefotaxime and 20 µM $AgNO_3$. The medium was changed at 10–14 d interval. Within 2–4 weeks, the shoot primordia emerged, and the explants were then transferred to regeneration medium containing 25 mg/l kanamycin, 250 mg/l cefotaxime, and 20 µM $AgNO_3$. The explants were placed in fresh medium at intervals of 10–12 d. Green, putative-transformed shoots were repeatedly transferred to fresh hormone-free MS medium containing 25 mg/l kanamycin and 200 mg/l cefotaxime.

GUS activity was tested histochemically in various plant parts like leaf, root, stem, sepal, petal, anther, pistil, pollen, pod of different transgenic plants as well as their subsequent progenies. The histochemical determination was carried out according to the procedure

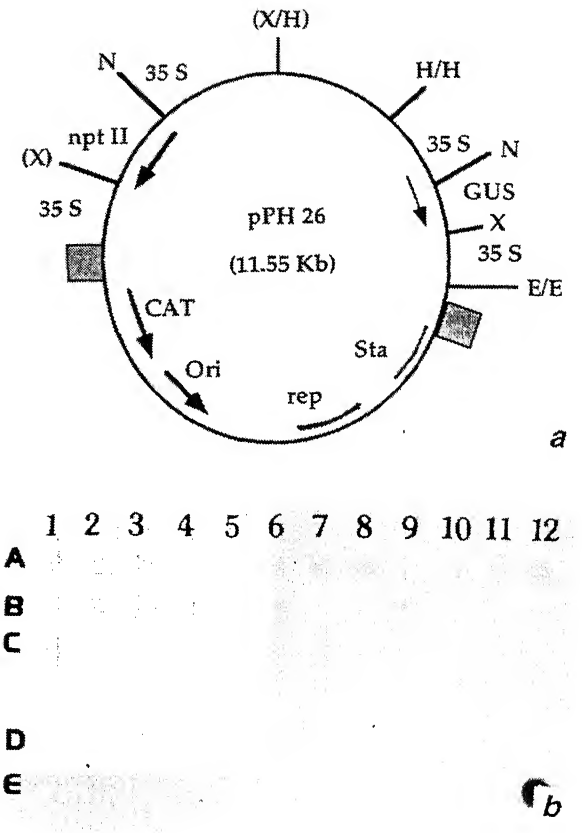

Figure 1. pPH26 construct and dot blot for molecular characterization of the transgenics: *a*, Construct used in transformation experiment (pPH26); *b*, Dot blot using *npt*II gene as marker probe. Lane A; 1–12, all putative transformants. Lane B; 1–12, all putative transformants. Lane C; 1–6, all putative transformants along with negative control (untransformed plant) at C_7 position. Lane D; 1–6, putative transformants along with negative control at D_7 position. Lane E_{12}; positive control.

of Jefferson[5]. Tissue sections or organs were incubated overnight at 37°C in x-gluc solution made up of 10 mM EDTA, 100 mM sodium phosphate buffer (pH 7.0), 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, and 0.1% (w/v) x-gluc. Chlorophyll was removed by treating the tissue in 1:3 aceto-ethanol mixture. A blue colour was observed in 70% ethanol in transformed shoots in comparison to white colour in untransformed control plant.

The green and healthy rooted plants were transferred to soil for acclimatization after proper washing away of agar from the root surface. The potted plants were initially covered with a plastic bag, for 7–10 d and later slowly exposed to the ambient environment of the glasshouse at 20–22°C with 60–70% humidity and natural photoperiod.

The presence of transgene (*npt*II) was detected through dot blot analysis. Plant DNA was extracted

using urea buffer according to the method of Sambrook *et al.*[6]. The final pellet was treated with RNAase and dissolved in Tris–EDTA buffer. The total genomic DNA was blotted on nitrocellulose membrane after denaturing the DNA by treating with 4 M NaOH. Pre-hybridization and hybridization procedures were followed according to Sambrook *et al.*[6]. The probe was prepared using *npt*II gene cut from the vector, labelled with [α-^{32}P]dATP (Amersham), using PRIME IT TM random primer kit from Stratagene. After hybridization, washing was carried out under high stringency condition (65°C, 0.1 × SSC, 0.5% SDS).

Among the different media tested for regeneration from hypocotyl explants, 0.01 mg/l 2,4-D and 2.0 mg/l BA were found to be the best hormone combination (9.0% regeneration). Use of 20 µM $AgNO_3$, an ethylene inhibitor, increased the plant regeneration to 31.8%. Additionally, when 0.7% agarose was used as the gelling agent instead of agar agar, the plant regeneration frequency increased to 71%.

The ability of hypocotyl tissues to regenerate shoots was significantly reduced by infection with *Agrobacterium*, and was delayed by 5–6 d. Presence of kanamycin (25 mg/l) in the selection medium had adverse effect on shoot induction. Hence, kanamycin selection was applied after 10–14 d of culture after induction of shoot primordia. Two types of shoots, green and white with occassional purple pigment in the young leaves were produced on kanamycin-containing medium. The green shoot buds were considered to be the resistant, and putative transformants. The green shoot buds were repeatedly transferred to fresh kanamycin (25 mg/l)-containing, hormone-free MS medium. From two different sets of experiments it was observed that all the green shoots raised from the kanamycin-containing shoot-induction medium could not produce roots in the same selection medium. Some turned white, while others showed rooting but turned white after several passages, and did not produce healthy green plants. In the first experiment, 13 out of 25 selected shoots (52%) and in the second experiment 24 out of 43 selected shoots (57%) of the regenerated green shoots showed rooting and normal growth in further selection medium. The transformation frequency was calculated as:

$$\frac{\text{No. of explants produced transgenic shoots} \times 100}{\text{No. of total explants used in the experiment}}.$$

The calculated transformation frequency was 3.2 and 5.0% in the two independent experiments (Table 1).

The plant DNA was isolated from the leaf tissue of all kanamycin-resistant plants together with the negative control. One-kb fragment of *npt*II gene was used as the probe to check for presence of the *npt*II gene among the selected plants. Among the 36 selected plants tested, 24 showed clear presence of *npt*II gene; negative control did not hybridize in the same blot (c 7 and d 7 position in Figure 1 *b*).

Histochemical assay for presence of *gus* gene was carried out in all the putative transformants; small pieces of leaves from each plant were used for the assay. The experiment revealed that many of the transgenic shoots were chimeric in nature. The few constitutively expressing GUS-positive plants were tested for the

Table 1. Transgenic plants of *Brassica juncea* using pPH26 construct containing *gus* reporter gene and *npt*II marker gene

Number of	Expt. no. 1	Expt. no. 2
Explants cultured	156	168
Explants that developed green shoots	16	28
Green shoots recovered	25	43
Transgenic shoots	13	24
Explants that produced transgenic shoots	5	
Transformation frequency	3.2%	5.0

Table 2. GUS expression in different parts of transgenic plants

Plant parts	Positive (+)/Negative (–)
A. Root	
(i) germinating seedling root	
(a) apical region	++
(b) other regions	–
(ii) mature root	
(a) apical region	++
(b) other regions	+
B. Stem	
(a) epidermis	+
(b) cortex	+
(c) vascular bundle	++
(d) pith	–
C. Leaf	
(a) young leaf	++
(b) mature leaf	
(i) margin/tip	++
(ii) vascular region	+
(iii) petiole	–
D. Flower	
(a) sepal	++
(b) petal	
(i) vascular region	+
(ii) other regions	–
(c) androecium	
(i) filament	–
(ii) anther lobe	++
(d) gynoecium	
(i) ovary	–
(ii) style	+
(iii) stigma	++
E. Fruit	
(a) pod wall	+
(b) funicle	++
(c) mature seed coat	–
F. Cotyledonary leaf	++

++, indicates dark blue; +, indicates light blue.

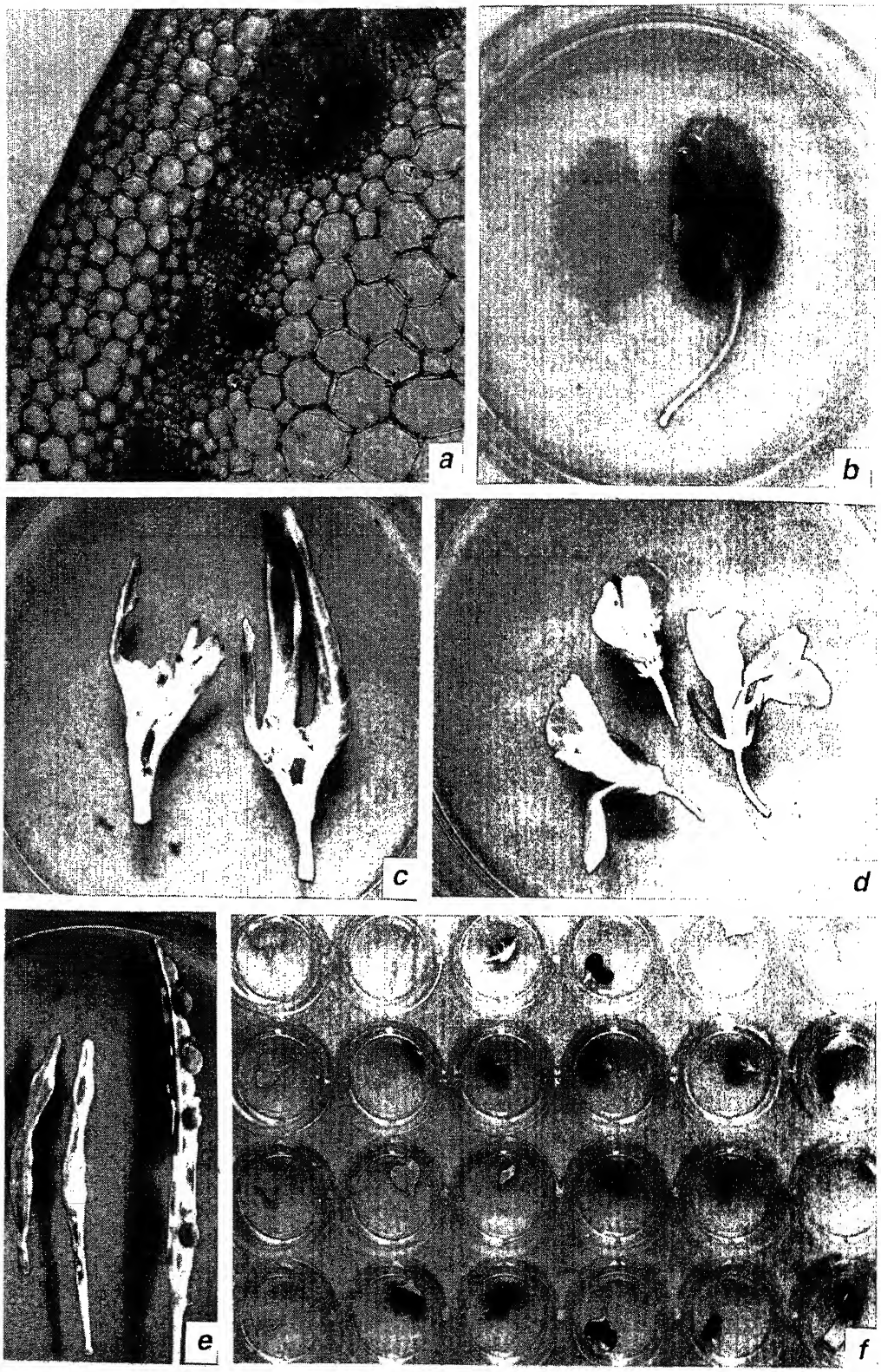

Figure 2. Histochemical assay for presence of *gus* gene in transgenic plants. (*a–e*, T_0; f : T_1). *a*, Stem section showing *gus* gene expression in the cortical region and vascular tissue (× 120); *b*, Young leaf of transgenic plant showing GUS expression (right) and the young leaf from control plant not showing any GUS expression (left) (× 1); *c*, Inflorescence axis of transgenic plant bearing flower buds (FB) and young leaves (YL), all showing GUS expression (× 2); *d*, Flowers of transgenic plant showing *gus* gene expression in sepals (S), petals (P), anther lobes (AL), style and stigma (SS) (× 2); *e*, Pods bearing the seeds showing presence of *gus* gene expression on the pod wall (PW) and funicles (F) (× 1.5); *f*, Segregation of *gus* gene evidenced by blue coloration of cotyledonary leaves in a small population of T_1 progeny when stained with x-gluc (× 1).

presence of *gus* gene in different plant parts (Figure 2 *a–e*). Table 2 gives the GUS expression in different organs of transgenic plants.

Seeds were obtained from the bagged inflorescences of three different nonchimeric transgenic plants which were transferred to soil. On germination in kanamycin (50 mg/l)-containing, hormone-free MS medium; the selfed seeds showed segregation of resistant (green) and sensitive (white) seedlings. The statistical data given in Table 3 prove that the gene for kanamycin resistance showed dominant, monogenic Mendelian inheritance. In addition, all the seedlings were tested histochemically for the presence of *gus* gene which was found to be present in all the kanamycin-resistant seedlings, but was absent in all the kanamycin-sensitive seedling (Figure 2*f*). The experiment proved the co-segregation of both the marker and the reporter genes. Some of the green seedlings of the T_1 generation were transferred to pots to produce the T_2 seeds. GUS expression was examined in different plant parts like flowers, seeds, pods, etc. in the T_1 plants. The flowers showed the same expression as in T_0 transgenic progeny (Figure 2*d*). When a random population of T_1 transgenic plants was maintained in culture in presence of 100 mg/l kanamycin, after prolonged culture in kanamycin-containing media the plants showed differential growth pattern: out of 20 plants, 4 showed deep green colour in leaf and normal growth, but the other 16 showed pale appearance with normal growth and proper rooting. Potrykus *et al.*[7] had reported similar segregation for dark and light green leaf colour in transgenic tobacco plants with the *npt*II gene, which the authors attributed to dosages effect of the transgene. After 8 weeks of *in vitro* culture in kanamycin-containing medium, *gus* gene expression was not observed in 25% of the plants that were checked histochemically. Occasional instability or loss of the marker gene in the progeny has been reported by Potrykus *et al.*[7].

The T_2 seeds (selfed seeds) of the transgenic plants were obtained from the T_1 plants. The seeds from two

Table 3. T_1 progeny analysis of *B. juncea* transgenic plants developed through *Agrobacterium*-mediated gene transfer of pPH26 plasmid containing *gus* reporter gene and *npt*II marker gene

Plant no.	T_0-1	T_0-22	T_0-31
No. of seeds tested	56	81	68
No. of seeds germinated	27	50	38
No. of plants that showed gus-positive reaction	20	38	29
Expected no. of GUS-positive plants according to Mendelian 3:1 ratio	20.25	37.5	28.5
*Chi square value	0.0122	0.0266	0.0241
Kanamycin positivity test	All GUS positive plants showed karamycin positivity at 50 mg/l kan		

*Probability lies between 80 and 95%.

Table 4. T_2 progeny analysis of two different T_1 transgenic *B. juncea* plants

Plant no. T_0-22-4	
No. of seeds tested	176
No. of seeds germinated	160
No. of plants that showed kan resistance at 100 mg/l kan	124
Expected no. of kan^r plants according to Mendelian 3:1 ratio	120
*Chi square value	0.533
Plant no. T_0-22-5	
No. of seeds tested	201
No. of seeds germinated	196
All the plants were kan resistant and could tolerate up to 200 mg/l kan	

*Probability lies between 50 and 20%.

different T_1 plants were germinated in the presence of 200 mg/l kanamycin. The seeds from one plant (T_0-22-4) showed the segregation of green and white seedlings in a ratio of 3:1 (Table 4) when germinated in the selection medium, indicating that the particular plant was heterozygous in respect of marker gene in T_1 generation. All the seedlings from another plant (T_0-22-5) remained green, and did not show segregation. Hence the plant T_0-22-5 of T_1 generation was homozygous in respect of the marker gene. When all these plants were tested in 300 and 500 mg/l kan, they all showed proper rooting in both the concentrations, but few of them bleached. When GUS expression was checked from a random population, it showed co-segregation of both the genes.

This report of transformation of Indian mustard, an important oil seed crop of this subcontinent, with the *gus* reporter gene and its stable inheritance in 2nd and 3rd generations is an essential step towards transfer of agronomically useful gene(s). Here the protocols for transferring foreign genes into *Brassica juncea* at a high transformation frequency have been developed that can be utilized further for introgression of agronomically important gene(s).

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ACKNOWLEDGEMENTS. We thank Council of Scientific and Industrial Research, India, for providing financial assistance in the form of a Research Fellowship to S.P. during the work.

Received 28 October 1998; revised accepted 19 March 1999

Detection of sandal spike phytoplasma by polymerase chain reaction

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Spike disease affected sandal (*Santalum album* L.) tissues were screened for the presence of the pathogen, a non-culturable phytoplasma using polymerase chain reaction (PCR) technique. Oligonucleotide primers specific to the conserved region of 16S rRNA gene were used to amplify a 558 bp sequence of the phytoplasma. Four DNA fragments were obtained when the PCR products after 20 cycles of amplification were subjected to restriction fragment length polymorphism analysis (RFLP) with *Alu*I restriction endonuclease. The technique confirms that sandal spike phytoplasma belongs to group I of the eleven major phytoplasma groups.

SPIKE disease, the major disease in sandal (*Santalum album* L.), is caused by a non-culturable phytoplasma seen exclusively in the phloem tissues[1]. Detection of the pathogen was mainly through electron microscopy[2–4] or indirect methods using different stains like aniline blue[5] and Giemsa[6] stain by light microscopy. Recently, the DNA binding fluorochrome, 4,6 diamidino-2-phenylindole (DAPI) was employed for the detection of the pathogen[7]. Since phytoplasmas are associated with diseases of many plant species[8], neither of these methods allows the differentiation of the organism and its classification[9].

Studies of DNA homology in the highly conserved genes encoding ribosomal RNA and ribosomal protein have shown that phytoplasmas comprise a coherent set distinct from other prokaryotes[10,11]. A system for classification of phytoplasma based on amplification of the 16S rDNA by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) was introduced by Ahrens and Seemuller[9]. Since then, several groups of phytoplasmas have been differentiated on the basis of nucleotide sequence variation in 16S rRNA genes[11–13]. Sinclair *et al.*[14] have classified phytoplasmas into eleven major taxonomic groups based on the 16S RFLP fragments. In this paper we report the detection of phytoplasma from spike disease affected sandal by PCR using group specific primer followed by RFLP analysis.

Spike disease affected sandal was collected from Marayoor, Munnar Forest Division, Kerala and Chamundi Hills, Mysore, Karnataka. Total DNA samples from healthy and diseased plants were extracted following the modified protocol of Doyle and Doyle[15]. One gram tissue of midrib and young stem was ground into fine powder using liquid nitrogen. Two ml of hot (65°C) cetyl trimethyl ammonium bromide (CTAB) buffer (2% CTAB, 100 mM Tris, pH 8.0; 20 mM EDTA, pH 8.0; 1.4M NaCl) was added to the powder and samples incubated at 65°C for 1 h. The samples were extracted with chloroform/isoamyl alcohol (24:1) followed by low speed centrifugation (1600 g) for 5 min and aqueous phase eluted out. To this was added 1/10 volume 3M sodium acetate (pH 5.2) followed by the addition of 2 volumes of cold (–20°C) absolute alcohol. After incubation at –20°C for 12 h, the DNA precipitate was centrifuged for 10 min at low speed. Ethanol (95%) was added to the DNA pellet and recentrifuged at the same speed. The supernatant was discarded, air dried and the pellet dissolved in 100 µl sterile distilled water. The total DNA was estimated at A_{260} (1 O.D = 50 µg ml^{-1}).

The method followed by Ahrens and Seemuller[9] that allowed the amplification of a 558 bp fragment of the 16S rRNA gene of phytoplasma was adopted for PCR with slight modification. The primers were designed from the conserved regions of the 16 S rRNA gene of O-MLO[16] located between 759 and 1359 bp. The sequence of the forward primer is 5′-ACGAAAGCGTG-GGGAGCAAA-3′ and the reverse primer is 5′-GAAGTCGAGTTGCAGACTTC-3′. A total volume mixture of 50 µl contained 1 µl of test DNA (200 ng), 1 µl of each primer (Bangalore Genei, India), 2.5 mM each of four dNTPs, 1 µl (3 units) of *Taq* polymerase (Bangalore Genei, India) or Dynazyme II (Finnzymes Oy, Finland) and 5 µl *Taq* buffer or Dynazyme buffer. The mixture was covered with two drops of mineral oil and subjected to 20 amplification cycles (PTC-150 Minicycler, MJ Research, USA) each of 30 s denaturation (95°C), 30 s annealing (55°C) and 30 s extension (72°C). The final extension step was for 5 min.

The PCR amplification products obtained from DNA of healthy and diseased sandal after 20 cycles were electrophoresed in 1.5% horizontal agarose (Sigma, USA) gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The gel was stained with ethidium bromide and bands compared with a 100 bp DNA ladder (Bangalore Genei, India). 15 µl of the reaction mixture obtained using *Taq* polymerase was digested with 1 µl of undiluted *Alu*I (Bangalore Genei, India) following manufacturer's instruction at 37°C for 12 h. 10 µl of the digest was used to resolve the restriction fragments on a 3% horizontal agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0), and the bands were visualized after staining with ethidium bromide. The gels were documented using Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, USA).

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After 20 amplification cycles, the DNA fragment of 558 bp was amplified from samples which contained DNA extracted from diseased sandal but not from healthy samples or water control. Bands were visualized for both *Taq* polymerase and Dynazyme II polymerase (Figure 1). On comparing the spike diseased tissues from Kerala and Karnataka, the bands appeared to be identical (Figure 2). Restriction analysis of the amplified fragment with *Alu*I revealed the presence of a pattern characterized by restriction sites at position a, b, and c with fragments of 240, 191, 71 and 56 bp (Figure 3 *a*, *b*), as described by Ahrens and Seemuller[9] for the first group of phytoplasmas.

Since phytoplasma cannot be cultured under axenic conditions, their identities and taxonomic position were unclear or uncertain until recently, when methods of molecular genetics were introduced into plant mycoplasmology[17]. The 16S rRNA gene, is a universal character which provides valuable phylogenetic and taxonomic information on culturable mollicutes and other prokaryotes[9,10,13]. Hence, this gene has widely been used to obtain phylogenetic information on non-culturable phytoplasmas. RFLP analysis of the amplified PCR product (rDNA) could distinguish various groups and subgroups of phytoplasma[17].

PCR method of detecting phytoplasmas gained popularity after the classical work of Ahrens and Seemuller[9]. The primers used were designed from the conserved regions of the 16S rRNA gene of the *Oenothera* phytoplasma (O-MLO). Phytoplasmas infecting several plants collected from different continents maintained in *Catheranthus roseus* for several years were used for their study. The mollicutes have a high mutation rate resulting in many totally unique oligonucleotides[18]. In our study, we used young stem and leaf midrib tissues from diseased and healthy sandal for isolating DNA, since the greatest yields of DNA will always be obtained using the youngest, freshest tissue. The liquid nitrogen

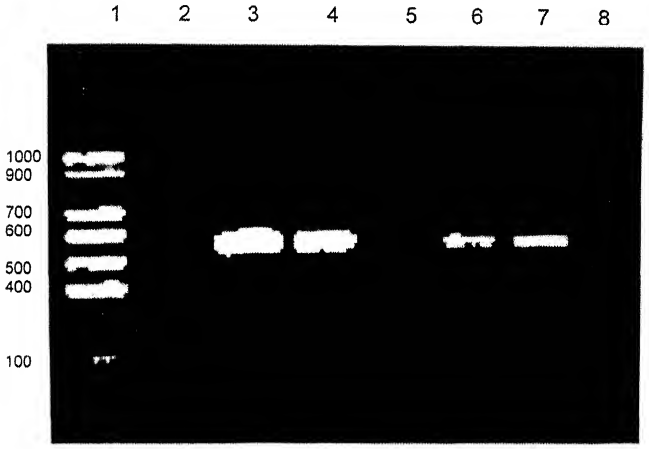

Figure 1. Agarose gel electrophoresis of polymerase chain reaction product (16S rDNA fragment ~558 bp) of sandal spike phytoplasma after 20 cycles of amplification using both *Taq* polymerase (lanes 2–4) and Dynazyme II (lanes 5–7). Lane 1, 100 bp ladder; lanes 2 and 5, healthy sandal; lanes 3, 4, 6 and 7, diseased sandal; lane 8, water control.

Figure 2. Agarose gel electrophoresis of polymerase chain reaction product (16S rDNA fragment ~558 bp) of sandal spike phytoplasma after 20 cycles of amplification using Kerala (lanes 3–5) and Karnataka (lanes 6–8) sandal spike infected tissues. Lane 1, 100 bp ladder; lane 2, healthy sandal.

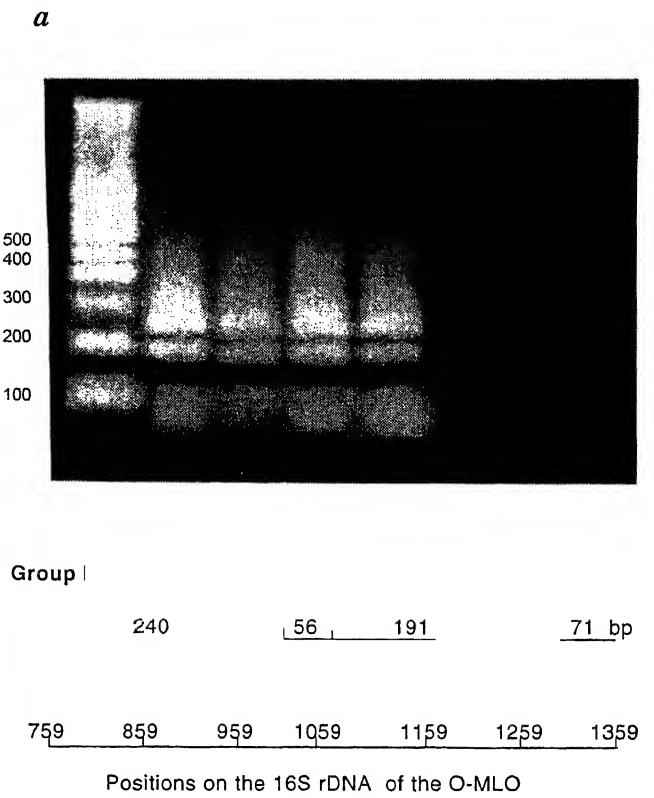

Figure 3. *a*, Agarose gel electrophoresis of *Alu*I digests of PCR products of sandal spike phytoplasma. Lane 1, 100 bp ladder; lanes 2 and 3, sandal spike phytoplasma of Kerala; lanes 4 and 5, sandal spike phytoplasma of Karnataka; *b*, *Alu*I restriction maps of the 558 bp 16S rDNA fragments of group I phytoplasma (Ahrens and Seemuller[9]).

powdered tissues were incubated at 65°C for 1 h for larger DNA yields[19]. 3M sodium acetate was used to induce DNA precipitation[20]. The yield of DNA pellet improved after incubating overnight at –20°C. The DNA pellet was dissolved in sterile distilled water and not in Tris–EDTA buffer since the same can interfere in PCR amplification, as EDTA was found to effectively reduce the magnesium concentration[21].

In our experiment we used both *Taq* polymerase and a DNA polymerase from *Thermus brockianus* (Dynazyme II) for PCR amplification. Both the polymerases amplified the phytoplasma DNA (Figure 1). In the subsequent studies only *Taq* polymerase was used and a 558 bp fragment of DNA was amplified from both the Kerala and Karnataka sandal spike populations (Figure 2). The last extension step was prolonged to 5 min to ensure that all the PCR products are of full length[22].

The restriction enzyme *Alu*I has the recognition sequence 5′-AG/CT-3′. The restriction enzyme was incubated with the PCR product overnight at 37°C rather than for 2 h to increase the efficiency during further detection by electrophoresis. As the PCR product in the study was 558 bp, TAE buffer and 1.5% agarose gel were used since the gel had an effective resolution in the range 3–0.2 kb (ref. 23). TBE buffer and 3% agarose gel were employed to visualize smaller DNA fragments less than 0.2 kb, while TBE buffer provided good resolution than TAE buffer[24].

The RFLP analysis with *Alu*I restriction endonuclease confirms that sandal phytoplasma could be attributed to the first group of phytoplasmas as proposed by Ahrens and Seemuller[9]. Different workers have identified phytoplasmas of the first group associated with diseases in lettuce[25], declining apricot[26], pear decline[27], dieback in papaya[28], periwinkle[29], corn poppy[30], etc. The same technique could be used to detect and classify phytoplasmas affecting different plants in India.

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ACKNOWLEDGEMENTS. The authors thank the Department of Biotechnology, Govt. of India for financial support. S.T. acknowledges CSIR, Govt. of India for a senior research fellowship.

Received 30 December 1998; revised accepted 22 March 1999

Total phenol profile in some rice varieties in relation to infestation by Asian rice gall midge *Orseolia oryzae* (Wood-Mason)

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Estimation of total phenols at the stem bases in genetically related rice varieties in relation to the gall midge infestation revealed the role of phenols in expression of plant resistance involving hypersensitive reaction against the insect conferred by the gene *Gm2*. However, their role in other genetically diverse rice varieties with gall midge resistance was not clear.

THE Asian rice gall midge (RGM), *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae), is a serious pest of rice in several rice-growing countries of Asia, including India causing an estimated annual yield loss of over Rs 300 crores[1,2]. Upon hatching from eggs, laid on rice plants, maggots crawl down the plant between leaf sheaths to reach the apical meristem on which they feed. Maggot feeding causes formation of a tubular sheath gall called silver shoot. Further differentiation of the affected tiller is arrested and the tiller is rendered sterile. Pupa wriggles up the elongated gall and drills an exit hole that allows emergence of the adult fly. The main approach for the management of this pest has been through the development of resistant varieties. Though the mechanisms of resistance in rice against RGM are not fully understood, the level of resistance is generally complete immunity. No distinct oviposition preference is exhibited among resistant and susceptible rice accessions[3]. Early work suggested morphological features of rice plant contributing to the poor establishment of maggots in resistant cultivars[4], while later studies noted that maggots were present in equal numbers in both resistant and susceptible varieties[5]. A predominant antibiosis component leading to mortality of 1st instar maggots has been observed by many workers[6,7]. Some of the RGM-resistant rice genotypes express, subsequent to the pest attack, hypersensitive reaction (HR) which involves tissue necrosis at the site of insect feeding and leads to maggot mortality[8]. Other resistant varieties do not express HR but maggot mortality is noted as in the former group. These two groups are referred to as HR+ and HR– types, respectively.

Higher concentration of phenols in shoot apices of gall midge-resistant rice varieties Shakti, Leuang 152 (ref. 9), Ptb 18 (ref. 10), IET 7008, IET 7009 and Siam 29 (ref. 11) have been reported without any regard to the pest infestation. However, analysis of either the basal stem portion or the whole plant sample of twenty-nine RGM-resistant and susceptible rice varieties for total phenol content did not reveal any correlation with resistance[5]. Reddy[12] noted an increase in the total phenol content following RGM infestation both in resistant and susceptible rice varieites. Thus a clear association has not been shown so far to indicate that phenols play a role in RGM resistance. Since genetically diverse resistance mechanisms against RGM exist in rice varieties and some of these are induced by the pest attack, the role of phenols in RGM resistance, if any, needs to be studied under known genetic background and with reference to the pest attack. Here we report the total phenol profile in genetically heterogeneous and homogeneous plant materials in relation to RGM infestation and establish a clear role of phenols in conferring RGM resistance in HR + genotypes.

All the plant materials were obtained from the collection of RGM-resistant rice germplasm maintained at the Directorate of Rice Research, Hyderabad. Rice varieties, selected on the basis of the reaction, genetics and nature of resistance[13], like Phalguna with *Gm2* gene (HR+ type) resistant to RGM biotypes 1, 2 and 5; W 1263 with *Gm1* gene (HR– type) resistant to biotypes 1, 3 and 5; Suraksha deriving resistance from Ptb 18 (gene undefined) (HR+ type) resistant to biotypes 1, 2, 3 and 4 and TN1 lacking any resistance gene, formed the genetically heterogeneous set. Another set of rice varieties, based on the RFLP data pertaining to the 47 recombinant inbred (RI) lines in F5 or F6 generation from the cross Phalguna/ARC 6650 (RP 1579) generated with 48 single copy DNA probes distributed over all the 12 chromosomes[14], with three resistant and four susceptible RI lines were identified which displayed 33–97% genetic homogeneity (Table 1). Plants were grown in plastic trays as rows of 20–25 seedlings and exposed to gall midge biotype 1 adults (50 females + 10 males) 8–10 days after sowing. The next day the trays were transferred to a humidity chamber (RH > 90%) for egg incubation. Eggs hatched on the third day of oviposition and this was treated as 0 day of infestation. Stem bases, 2–3 cm in length, were cut from 5 seedlings per replication, pooled and fresh weight recorded before phenol extraction in methanol at 60°C for 20 min. Five replications per variety were maintained. Plants were sampled on 0, 1, 3 and 5 days after infestation. Total phenol content was estimated following Price and Butler[15] and expressed as mg g^{-1} fresh tissue. Data were subjected to analysis of variance (ANOVA) and the mean differentiated by LSD at 5% using IRRISTAT 4.0 software.

Results of the first set of genetically heterogeneous plant material (Figure 1) did not indicate a clear trend

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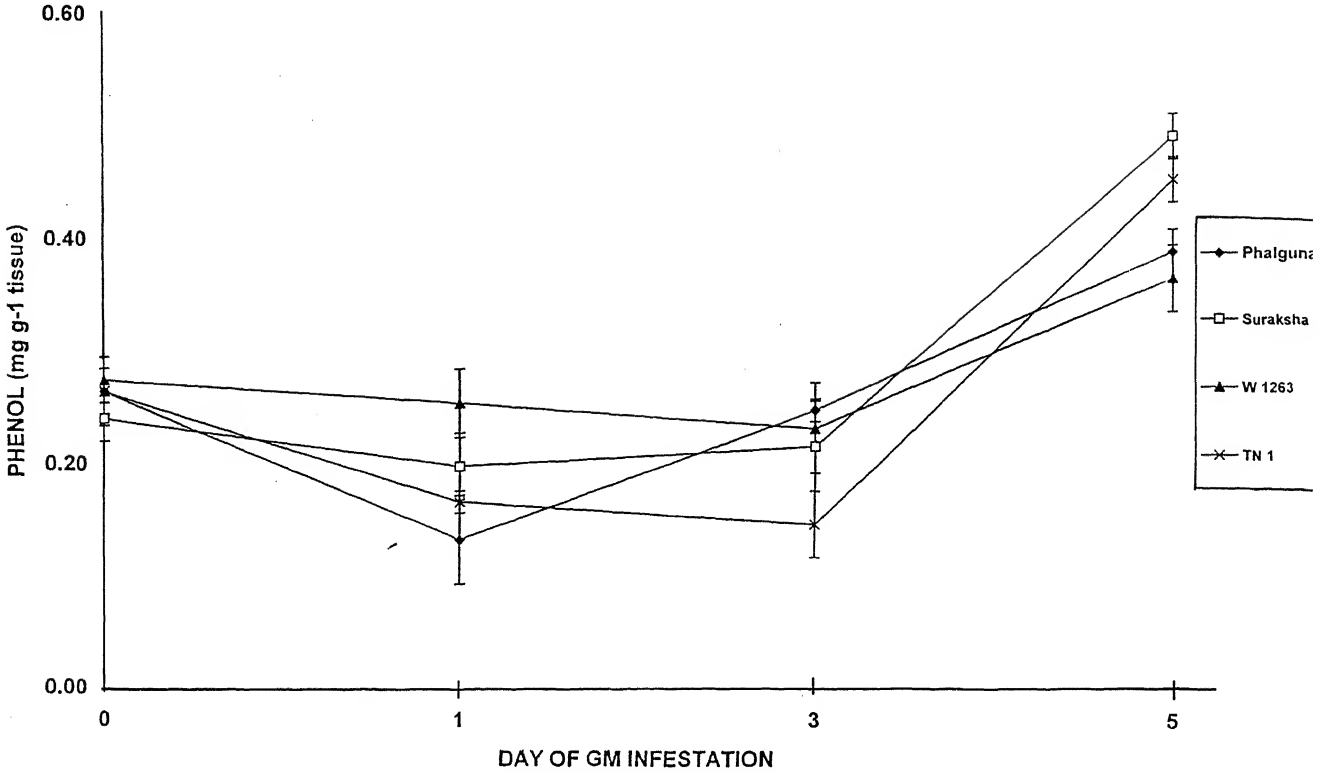

Figure 1. Total phenol profile at stem bases of genetically heterogeneous rice varieties in relation to rice gall midge infestation

Table 1. Genetic homogeneity among the recombinant inbred lines of the cross Phalguna/ARC 6650 (RP 1579)

RI line no.	Per cent homogeneity*		
	RI19 (R)	RI38 (R)	RI43 (R)
RI24 (S)	81	81	79
RI28 (S)	33	33	33
RI30 (S)	35	35	35
RI45 (S)	97	95	97

*Based on polymorphism data obtained with 48 RFLP probes[14].
R, resistant; S, susceptible.

among the varieties. The day of infestation had a significant effect on the total phenol content ($F = 63.5$, $P < 0.001$; $df = 3$) but effect of varieties was not significant ($F = 1.29$, $P > 0.05$; $df = 3$). Such an increase in phenol content could not be related to gall midge resistance since the susceptible control TN1 also showed this trend as noted earlier[12]. Phalguna variety registered significantly higher levels of phenol as compared to TN1 only on day 3 after infestation. In the second set, there was a clear trend of increase in phenol levels in all the three resistant RI lines, viz. RI19, RI38 and RI43. There was a significant day of infestation × variety interaction ($F = 5.42$, $P < 0.001$, $df = 21$). All the resistant lines recorded significantly higher levels of total phenols when compared to the susceptible lines, viz. RI24, RI28, RI30 and RI45 (Figure 2) on day 5 after infestation. Even 3 days after infestation RI19 and RI38 displayed increased levels of phenol compared with the susceptible lines. The tissue necrosis as part of HR is noted in Phalguna, a HR+ type donor, between day 3 and day 5 after infestation by gall midge biotype 1 (ref. 8). Thus increase in phenol levels between day 3 and 5 after RGM infestation in resistant RI lines and no such corresponding increase in the susceptible RI lines which share 95–97% genetic homogeneity with the former group can be conclusively related to RGM resistance mechanism conferred by *Gm2* gene.

A wide range of allelochemic compounds present in plants play an important defensive role against insects and other herbivores. Phenolics have been associated extensively with the chemical defense of plants against microbes, insects and other herbivores[16]. These compounds have the ability to form insoluble complexes with proteins, act as enzyme inhibitors or are oxidized to toxic quinones. Several associations have been reported between phenolics and the resistance of plants to insect damage[17]. Expression of HR, common in plant–pathogen interaction, involves phenyl–proponoid pathway[18]. Expression of HR as part of plant resistance against insects is rare but not uncommon[19]. Thus rapid

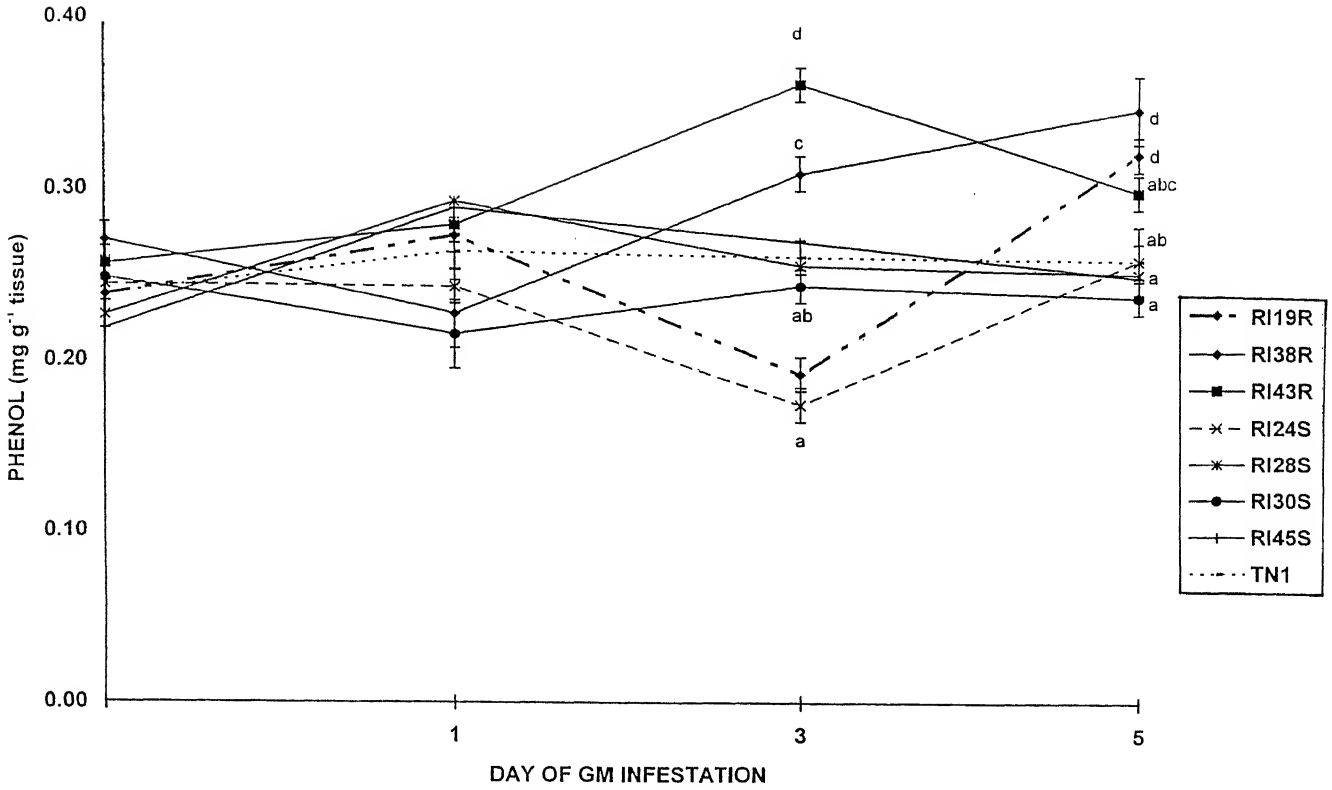

Figure 2. Total phenol profile at stem bases of genetically homogeneous rice varieties in relation to rice gall midge infestation.

accumulation of phenols in resistant RI lines following RGM infestation highlights the inducible biochemical pathway of expression of HR probably involving synthesis of phenolic precursors and their further oxidation into toxic quinones. It may be noted that once induced, the toxins produced are effective even against a virulent RGM biotype[8]. However, such a mechanism is not involved in the rice variety W 1263 used in the first set. A constitutive expression of a toxin of a different nature may be operative here against RGM. Such a diversity of expression of resistance against RGM among rice germplasm may have been the primary source of ambiguity in earlier reports. Slight increase in phenol content even in susceptible varieties may be associated with gall initiation process[20]. Phenols act as IAA oxidase inhibitors resulting in hyper-auxinity in gall tissue that leads to formation of nutritive tissue on which the gall formers feed. Thus both compatible and incompatible interactions in rice gall midge appear to follow an initial common biochemical pathway as has been highlighted in plant–microbe interactions[21].

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Received 31 December 1998; accepted 4 March 1999

Distribution of membrane-bound calcium and activated calmodulin in cultured protoplasts of sunflower (*Helianthus annuus* L.)

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Cultured protoplasts, isolated from the hypocotyl segments of seedlings of *Helianthus annuus*, exhibit rapid changes in intracellular-bound calcium and calmodulin (CaM) activation, in response to auxin (IAA, 10^{-5} M) treatment. Activities of bound calcium and CaM have been localized photomicroscopically, using specific fluorochromes – chlortetracycline (CTC) and trifluoperazine (TFP), respectively. Bound calcium accumulation is followed by an increase in Ca^{2+}–CaM activity. Bound calcium initially shows preferential accumulation in the nucleus, within 2 min of incubation of protoplasts in IAA-containing medium. The fluorescence gradually increases along the plasmalemma. Ca^{2+}–CaM activity shows similar but later (within 10 min of incubation) distribution in the cultured protoplasts. In the multicelled bodies, however, Ca^{2+}–CaM activity appears to be preferentially localized in the meristematic region, whereas bound calcium shows more uniform pattern of distribution. The percentage of protoplast populations exhibiting the above-stated changes in the distribution of bound calcium and calmodulin activation, varied between 70 and 85 in different experiments and their repititions. This indicates the important role of calcium and calmodulin activation in the manifestation of polarity.

SUNFLOWER has proved to be a relatively difficult plant for protoplast culture. A number of genotypes have been investigated[1]. Particular attention has been paid to the first stage of protoplast culture, so that the steps for further development can be optimized. Divisions in cultured protoplasts, their oriented growth and subsequent differentiation are believed to be under the control of ionic fluxes[2]. Intracellular calcium is involved in a large number of physiological processes and many external stimuli result in changes in intracellular concentration and compartmentalization of calcium ions and calmodulin[3,4]. There is increasing evidence that Ca^{2+} participates in the initiation and maintenance of polarity in plant cells[4] . In the light of these observations, we have undertaken a study of the distribution of bound calcium during the initial stages of protoplast culture in sunflower, together with an analysis of the distribution of activated calmodulin (Ca^{2+}–CaM complex) because of its dominant role in the regulation of calcium metabolism and cell division.

Monitoring intracellular free Ca^{2+} poses many problems in plant cells and the success of loading the specific fluorochrome depends on the plant in question and also the fluorochrome being used. Chlortetracycline (CTC), which has been used in the present work to localize intracellular calcium, has a good cell permeability and easily loads into plant cells[5,6] but it localizes membrane-bound calcium. Activated calmodulin (Ca^{2+}–CaM) can be detected by the use of a group of CaM inhibitors (phenothiazines, such as trifluoperazine; TFP)) which bind specifically with activated calmodulin forming a Ca^{2+}–CaM–phenothiazine complex[7,8]. TFP has been used in the present work to study the distribution of activated calmodulin in the cultured protoplasts.

Hypocotyls from 7-day-old *in vitro*, dark-grown seedlings were used for aseptic, enzymatic isolation of protoplasts. Hypocotyl segments (1 gm fw) were sliced and incubated in 5 ml of enzyme solution in plastic steriplates for 16 h in dark at 30 ± 2°C. The mixture was shaken gently for 10 min at the end of incubation and filtered through 80 µm stainless steel mesh. The protoplasts thus released were pelleted by centrifugation at 80 g for 5 min and washed thrice in the isolation medium (IM). This procedure results in a protoplast population free from cell wall debris. The composition of IM and enzyme mixture are as follows: IM (gm l^{-1}): NaCl 18; KCl 0.4; $CaCl_2 \cdot 2H_2O$, 6.13; MES 0.7 (pH adjusted to 5.6).

Enzyme mixture: Macerozyme R10 0.2%; cellulase TC 0.1%; pectinase boerozyme 0.5%. The individual enzymes (Serva Fine Chemicals Co, Germany) were dissolved in IM. Glassware used for protoplast isolation were sterilized by autoclaving at 15 lbs psi for 15 min. The enzyme mixture was filter-sterilized using sterilized filter assembly (pore size: 0.22 µm).

The viability of isolated protoplasts was tested after 5 min of incubation in 0.01% fluorescein diacetate

For correspondence.

(FDA). The protoplasts were examined under UV, using a photomicroscope (Zeiss, Germany) equipped with a FITC excitation filter. The viable protoplasts fluoresced bright green in the presence of FDA. In order to determine the time of incubation for optimum protoplast yield, hypocotyl slices (1 gm fw per 5 ml of enzyme mixture) were incubated in dark at 30 ± 2°C for 8, 10, 12, 14, 16 and 24 h, respectively. The yield of purified protoplasts was determined using a haemocytometer after the routine steps of washing. For culture, the pelleted and purified protoplasts were resuspended in hormone-free liquid medium (LB medium; modified from Guilley and Hahne[9]). The mixture was poured into multiwell steriplates as drops and immersed in liquid LB medium. In order to determine the protoplast density for optimal plating efficiency, cultures were raised in agar droplets containing the following five protoplast densities: 6×10^4, 12.5×10^4, 25×10^4, 50×10^4 and 10^5 protoplasts per ml. All protoplast cultures were initially incubated in dark for 48 h and subsequently transferred to light (4.3 watt m^{-2}). The modified LB medium[9] consists of the following components (mgs $^{-1}$ l): $CaCl_2$ 440; $MgSO_4$ 738; KH_2PO_4 68; H_3BO_3 6.2; $MnSO_4$ 0.17; $ZnSO_4$ 0.28; $CoCl_2$ 0.024; $CuSO_4$ 0.0025; Na_2MoO_4 0.024; myoinositol 100; thiamine 1; pyridoxine 1; sucrose 20,000; mannitol 80,000; MES 700. pH of the medium was adjusted to 5.6 before autoclaving.

In order to observe rapid changes in intracellular bound calcium and calmodulin activation due to auxin (IAA) treatment, protoplast preparations were treated with 10^{-5} M IAA (filter-sterilized) in IM (containing 41.6 µM $CaCl_2$) for various durations, viz. 2, 5 and 10 min. After treatment, the protoplasts were pelleted by centrifugation at 80 *g* for 2 min, washed in IM (minus calcium) and resuspended in a drop of CTC (2×10^{-4} M) dissolved in IM (minus calcium). The bright yellow fluorescence was observed using a Zeiss fluorescence photomicroscope (BP 355-425/DM 455/LP460). Controls consisted of protoplasts incubated in minus IAA medium and in medium containing calcium ionophore A23187 (10 µM) in the absence or presence of IAA. For localizing the activated calmodulin, agar droplets containing the cultured protoplasts were treated with TFP (5×10^{-5} M dissolved in IM). The preparations were observed for reddish-yellow fluorescence due to the complex of TFP with Ca^{2+}–CaM complex photooxidized under UV, using Zeiss fluorescence photomicroscope (BP 365/DM 400/LP420). With both the fluorescent probes, observations were recorded within 1 min of incubation in the fluorochrome. The results were photographed on Fujicolor negative film (ASA 400).

Optimum protoplast yield (11×10^4 protoplasts gm^{-1} fw) was obtained after 16 h of incubation in the enzyme mixture (Figure 1). An incubation of 24 h results in the isolation and subsequent bursting of protoplasts. Based on these observations, all subsequent experiments have been undertaken with 16 h of incubation.

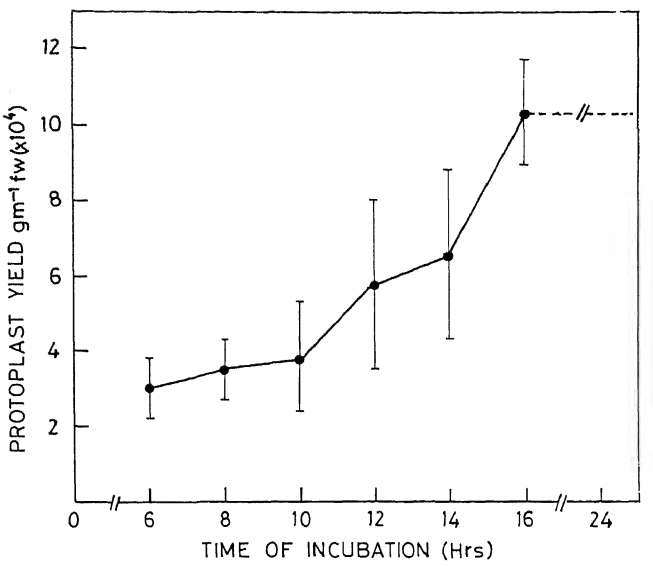


Figure 1. Protoplast yield from the hypocotyl segments of 7-day-old, dark-grown seedlings of *Helianthus annuus* L. as a function of time of incubation in the enzyme mixture. Data represent mean and standard errors from three observations.

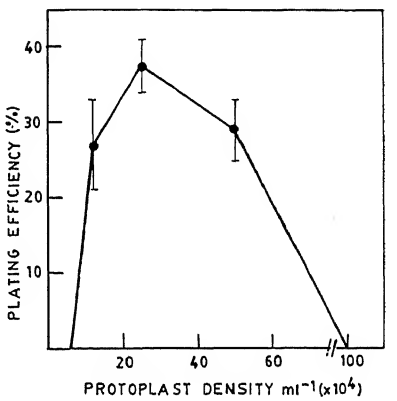
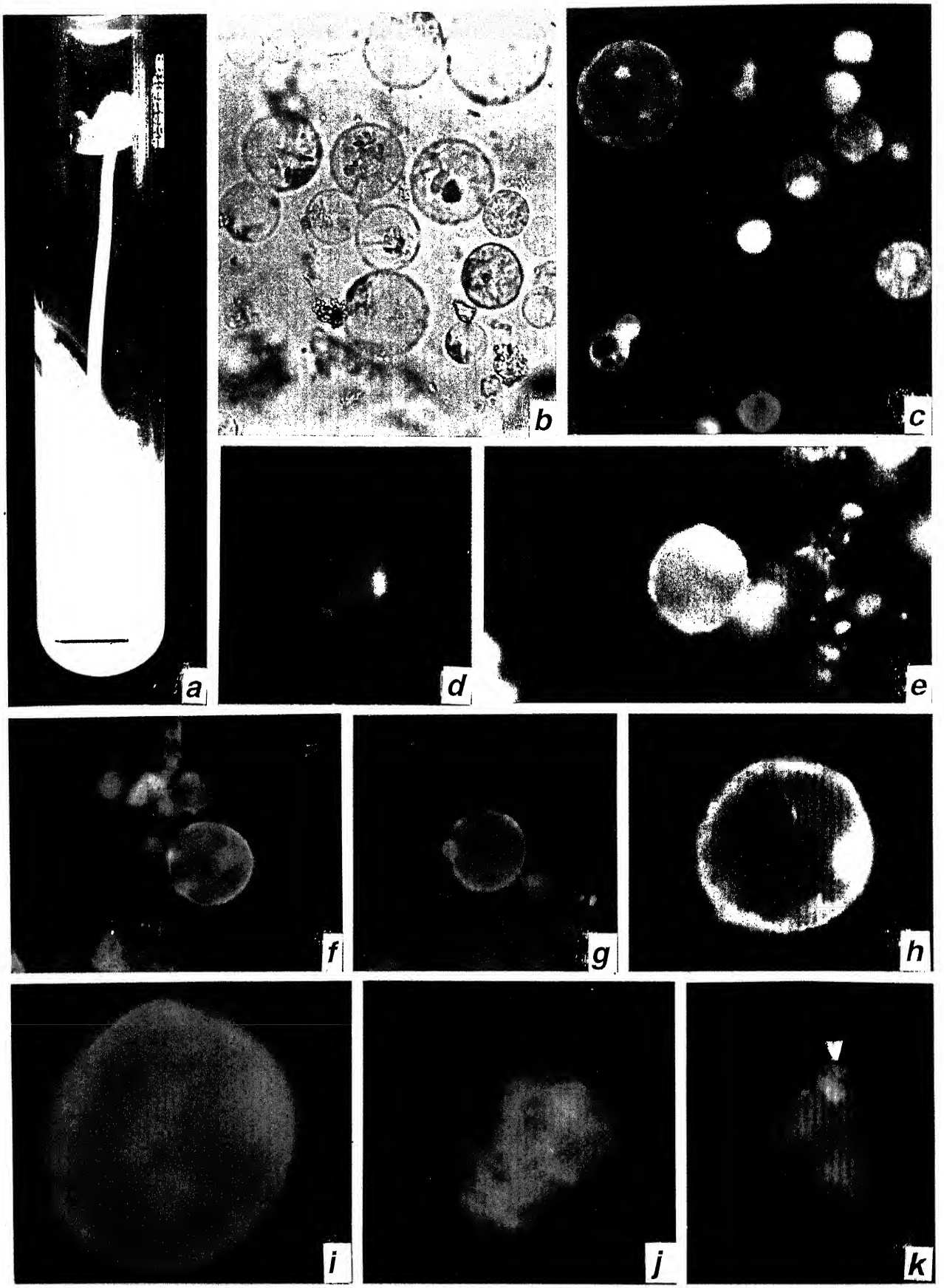


Figure 2. Effect of protoplast density on plating efficiency (%). Data obtained after 8 days of protoplast culture in agar (0.5%) prepared and bathed in LB medium. Data represent mean and standard errors from three observations.

Growth condition of the donor plant affects the yield of protoplasts. Hypocotyl slices from dark-grown seedlings (raised on MS medium) result in slightly better protoplast yield ($11 \pm 2 \times 10^4$ protoplasts gm^{-1} fw) when compared to those isolated from seedlings grown in light conditions mentioned earlier ($8 \pm 3 \times 10^4$ protoplasts gm^{-1} fw). A protoplast density of 25×10^4 ml^{-1} has been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found to be optimum for plating efficiency whereas the extreme populations do not promote any division (Figure 2). The protoplasts isolated from seedlings raised on MS medium and maintained at 25 ± 2°C in dark exhibit heterogeneity with regard to size, degree of vacuolation and presence of chloroplasts/plastids (Figure 3 *a, b*). Viability of the protoplasts is not affected significantly by light/dark conditions and it varies between 80 and 85% (Figure 3 *c*). IAA (2×10^{-6} M) remains ineffective in inducing protoplast divisions whereas at 10^{-5} M, it induces divisions in 18–20% protoplasts within 24 h. Further experiments on the localization of intracellular bound calcium and activated calmodulin have been done on protoplasts subjected to IAA treatment (10^{-5} M).

Since the fluorescence signal of CTC associated with membrane-bound calcium develops immediately upon exposure to UV and also decreases fast as a result of photobleaching, the observations were recorded within 1 min of irradiation. A longer exposure (5–7 min) leads to bursting of protoplasts. Calcium tends to accumulate within the protoplasts subjected to IAA treatment (10^{-5} M). IAA treatment for 2, 5 and 10 min results in an increase in fluorescence with time due to Ca^{2+}–CTC complex formation when compared with controls (–IAA; Figure 3 *d*). The fluorescence is localized more around the nucleus after 2 min of incubation and it also spreads along the plasmalemma (Figure 3 *e*). In 10 minutes, the fluorescence exhibits general cytoplasmic distribution (Figure 3 *f*). The effect of IAA is nullified by the co-incubation with calcium ionophore A 23187 (10 μM) for a period of 5 min (Figure 3 *g*).

The Ca^{2+}–CaM–TFP complex within the viable protoplasts results in an intense fluorescence immediately after UV irradiation. Prolonged exposure to UV leads to photobleaching of protoplasts and consequent decrease in the fluorescence intensity after 2–3 min. Observations were, therefore, recorded within 1 min of irradiation. Fluorescence development for Ca^{2+}–CaM complex was not as rapid as that for calcium. No signal could be detected in controls (–IAA) and in protoplasts incubated in the presence of IAA up to 2 min. In the protoplasts subjected to IAA (10^{-5} M) treatment for 10 min, fluorescence due to activated calmodulin is mainly concentrated along the plasmalemma and the nucleus (Figure 3 *h*). In contrast to this, bound calcium tends to show faster localization along the plasmalemma and also the cytoplasm (Figure 3 *e*). Longer incubation of protoplasts in IAA solution (20 min), however, results in an overall distribution of Ca^{2+}–CaM complex along the plasmalemma, nucleus and cytoplasm (Figure 3 *i*), as also observed in the case of bound calcium (Figure 3 *f*). But the observation is delayed when compared to bound calcium. Multicelled bodies formed as a result of protoplast divisions in IAA-containing medium, show a homogeneous distribution of bound calcium throughout the cells (Figure 3 *j*). Although both calcium and calmodulin activities show a general distribution all through the cells, Ca^{2+}–CaM activity is intense in the apical region (Figure 3 *k*). This is in contrast to the relatively uniform distribution of CTC-bound Ca^{2+} (Figure 3 *j*).

The present investigations reveal that hypocotyl slices from dark-grown seedlings result in a better yield of viable protoplasts than from light-grown seedlings and the viability varies between 75 and 85%. Earlier work has shown that sunflower protoplasts cultured in liquid medium produce colonies which fail to develop into calli[10]. Solidified medium provides better conditions for the development of colonies leading to compact microcalli formation. Plating efficiency of the protoplasts has been found to be affected by the density of the protoplast populations as well as the prevailing nutritional conditions. A varying proportion of the cultured protoplasts produce spherical microcolonies which usually develop into very compact structures, often with some apparent polarity. These structures do not surpass 20–30 cell stage and subsequently become necrotic or develop into microcalli[9]. Colonies from all types of genotypes so far investigated (including the present one), that are formed in agarose/agar, are either unorganized or dense, bipolar structures[9].

Incubation of protoplasts in IAA-containing medium results in marked changes in CTC and TFP fluorescence, indicating the mobilization of bound calcium and activation of calmodulin, respectively. CTC fluorescence is an indication of the amount of membrane-bound calcium. Earlier work using electron microscope, has shown an increase in the number of endoplasmic reticulum (ER) profiles in the outer, cytoplasm-rich cells from carrot proembryogenic masses[11]. The increased CTC signal in the present investigation might be in correlation with these results since ER is known to sequester up to millimolar levels of calcium[2]. The decrease in bound calcium signal in the presence of calcium ionophore A 23187 is due to the binding of Ca^{2+} to the ionophore at

Figure 3. Localization of intracellular-bound calcium and Ca^{2+}–CaM complex in auxin (IAA)-treated protoplasts of *Helianthus annuus* L. *a*, 7-day-old, dark-grown seedling raised on MS medium; *b*, Isolated protoplasts as observed in visible light (× 400); *c*, Viable protoplasts as observed in UV (× 400); *d–f*, Control (–IAA) medium. *d*, Fluorescence due to calcium CTC complex, after incubation in control medium (–IAA), *d*, 10^{-5} IAA for 2 min *e*, and 10 min *f*, (× 400); *g*, 5 min treatment with calcium ionphore A23187 (× 400); *h*, Fluorescence due to Ca^{2+}–CaM complex after subjecting the protoplasts to IAA (10^{-5}M) for 10 min (× 600); *i*, Protoplasts showing homogenous distribution of Ca^{2+}–CaM complex after 20 min incubation in +IAA medium (× 1000); *j*, Bound calcium distribution in multicelled bodies (× 400); *k*, Ca^{2+}–CaM distribution in multicelled body (× 400).

relatively low concentrations of extracellular calcium (41.6 μM). Present observations indicate a preferential accumulation of activated calmodulin in certain specific regions of the multicelled bodies, with the onset of polarity by auxin treatment. IAA has earlier been reported to promote calcium release from the membranes of mungbean and soybean hypocotyls and pea epicotyl[12]. IAA has, however, no effect on calcium uptake in wheat protoplasts[13] and hypocotyl segments of *Cucurbita pepo*[14]. Later work has, however, suggested calcium as a second messenger in early auxin action whereby IAA has been found to rapidly increase the intracellular free calcium levels in the cytoplasm[15–17].

The elevation of $[Ca^{2+}]$ in response to a signal may be uniform throughout a cell or a group of cells or it may be highly localized in certain specific regions of the cell. In many cellular systems, the calcium signal occurs as a wave, beginning at a discrete initiation site and then moving across the site[18]. The present work on *Helianthus* protoplasts shows that bound calcium is initially localized around the nucleus and later, as a result of continued auxin action, it tends to be preferentially localized along the plasmalemma as well. This may have something to do with the activation of calcium-binding sites along the plasmalemma and intracellularly. Intracellular calcium concentration can be increased rapidly by the transient opening of plasmamembrane calcium channels or by the release of calcium ions sequestered in ER. Both mechanisms are activated by the binding of extracellular signals to the plasmamembrane receptors. The elevated $[Ca^{2+}]$ within the cells binds to calmodulin, leading to an activation or inhibition of the target proteins.

Attempts to localize calmodulin during mitosis, using affinity-purified antibodies, have shown dense staining along the spindle poles, suggesting that calmodulin is involved in mitotic functions[19]. The present observations also show similar preferential localization of calmodulin along the nucleus, indicating its possible involvement in mitotic functions. Many other cellular functions have also been assigned to calmodulin through a combination of localization, biochemical and genetic approaches[20]. From the present comparison of fluorescence due to bound calcium and activated CaM in the protoplasts of *Helianthus*, it appears that membrane-associated Ca^{2+} may not be exclusively bound to CaM. Similar observations were also made in the proembryogenic cell masses of carrot[4]. Greater accumulation of fluorescence due to CaM in certain specified zones of the multicelled structures may indicate an important role for CaM in the manifestation of polarity. Calmodulin might act as a mediator of calcium flux across the membranes and ultimately as a sensor of cytoplasmic calcium transient[2]. Non-uniformity in the fluorescence due to bound Ca^{2+} and CaM in the protoplasts of *Helianthus* further indicates the possible participation of other calcium-binding proteins in relation to the calcium-dependent processes. Monitoring intracellular free Ca^{2+} poses many problems in plant cells and the success of loading the specific fluorochrome depends on the plant in question and also the fluorochrome being used. Attempts are underway to look into this aspect in the cultured protoplasts of *Helianthus*.

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ACKNOWLEDGEMENTS. The photomicroscope used in the present investigation was a kind donation from the Alexander von Humboldt Foundation (Germany) to S.C.B. as a Fellow of the Foundation.

Received 23 February 1999; revised accepted 6 April 1999

Truce with oxygen – Anaerobiosis outcompete aerobiosis in the Antarctic lacustrine bacteria

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The total number of bacteria counted directly by epifluorescent microscopy showed that they ranged from 10^8–10^9 l^{-1} in Antarctic lake water samples. The percentages of retrievable viable counts (RVC) of anaerobic bacteria (AnB) was greater than aerobic counts. Among the different groups of anaerobes, the order of retrieval was *Thiobacillus denitrificans* like organisms (TDLO) > fermentative bacteria (FB) > sulfate reducing bacteria (SRB). The total direct anaerobic viable counts (TDAnVC) was one order more than the total direct aerobic viable counts (TDAeVC). Laboratory experiments with one of the lake-isolates indicated that there was a tendency to express higher viability of 61% at redox potential (Eh) ranging from –281 to –335 mv. It is suggested that the disposition to express increased viability under reducing conditions is a strategy to counteract stress due to supersaturation of oxygen in the cold lacustrine environment.

STUDIES in the antarctic region have stressed on the biomass and activities in the terrestrial and aquatic ecosystems[1–3] and have pointed out that the bacteria-based food webs are as important in overall energy and material cycling in the high latitude oceans as they are at lower latitudes[4]. In the course of analysing antarctic water samples from lakes, the unusual phenomenon of retrievable viable counts (RVCs) of total anaerobic bacteria (AnB) far exceeding the aerobic ones in the form of colony forming units (CFUs) was noticed. The averages of general heterotrophic AnB were > *Thiobacillus denitrificans* like organisms (TDLO) were > lactate and acetate fermentors (FB) were > sulfate-reducing bacteria (SRB) were > aerobic bacteria (AB) in lacustrine environment where the dissolved oxygen is known to range from 10.4–13.8 mg l^{-1} (ref. 5). It has been a common observation that the retrievable aerobic counts in the form of CFUs are generally higher than anaerobic counts in surface layers of any water body. Exceptions are from specialized ecosystems like offshore oil wells[6] or deep, anaerobic, alkaline aquifers[7], where the anaerobic counts are higher than the aerobic. In the antarctic lake waters where the oxygen concentration is generally high, it was intriguing to encounter more anaerobic bacteria than aerobic forms in the surface waters. The importance of the anaerobic forms *vis-à-vis* the aerobic ones have however received little attention. SRB have been recovered from anaerobic bottom waters by Konda *et al.*[8].

Further, it was observed that the direct viable counts carried out in these water samples showed higher viability under reducing conditions. Does the observation reflect the physiological adaptation in bacteria to extreme conditions? This paper will discuss these findings along with laboratory experiments to corroborate the observations.

During the 13th Antarctic expedition (Dec. 1993–March 1994), microbiological sampling was carried out from Antarctic lakes (including lake Priyadarshini) around the Maitri Station. Water samples were collected in sterile Erlenmeyer flasks and stored in ice until analyses at field station, within 5–6 h of collection.

For total direct counts (TDCs), an aliquot of sample was immediately preserved with 2% formalin. The fixed sample was stored in the cold and further processed at the National Institute of Oceanography (NIO), Goa. Bacteria were estimated using the acridine orange direct count (AODC) method as described by Hobbie *et al.*[9] and the counts are expressed as number per litre.

Total direct viable counts (TDVCs), were estimated as outlined by Kogure *et al.*[10,11] using a mixture of piromedic, pipemedic, nalidixic acid and yeast extract. To differentiate anaerobic viability from aerobic, a reductant i.e. Na_2S at a final concentration of 0.0125% (125 ppm) was added before incubating the samples. These experiments were carried out in screw-capped tubes filled to the brim to minimize oxidation. As the tubes were incubated at low ambient temperatures of 8–12°C, the period of incubation was extended to 12–16 h. The samples were fixed and the numbers were estimated as described above.

For retrievable counts (RC), CFUs were counted on suitable media by spread plating. Plating was carried out within a few hours of collection. Nutrient agar prepared with freshwater was used to estimate the CFU of AB and agar shake tubes for AnB. The final volume of inoculum in the tube being as high as 5 ml in 15 ml screw-capped tubes and the concentration of agar only 0.8%, the heat shock to the microbes was minimal. In addition, other special media like modified Hatchikian's medium[12] and Leiske's medium[13] were used for enumerating specific anaerobic groups like SRB, FB and TDLO, respectively. All colonies that were not black by sulfide precipitation were counted as FB. The anaerobic CFU were AnB, FB, SRB, and TDLO. The plates were incubated at 8–10°C for 10–20 days and the tubes for ca 30 days. All samples have been analysed in replicates and only the average values of 20 samples from different lakes are presented.

A laboratory experiment was set up to find out whether the viability of bacteria is increased under

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Table 1. Aerobic versus anaerobic counts in the Antarctic lake water samples

Bacterial parameter	No. × 10^8 l^{-1} (epifluorescent counts)			No. × 10^4 l^{-1} (retrievable viable counts)				
	TDC	TDAeVC	TDAnVC	AB	AnB	FB	SRB	TDLO
Average (± SD) $n = 20$	20.45 (± 24)	1.57 (± 1.42)	18.12 (± 21.48)	0.11 (± 0.16)	16.23 (± 12.14)	1.19 (± 1.19)	0.27 (± 0.27)	5.08 (± 11.01)
Percentage of TDC		7.60	88.60	0.0001	0.01	0.001	0.0001	0.003

Microscopic counts – TDC, Total direct counts; TDAeVC, Total direct aerobic viable counts; and TDAnVC, Total direct anaerobic viable counts.
Retrievable plate/tube counts – AB, Aerobic bacteria; AnB, Anaerobic bacteria; FB, Fermentative bacteria; SRB, Sulfate-reducing bacteria; and TDLO, *Thiobacillus denitrificans*-like organisms.

reducing conditions at low temperatures. A gram negative psychrotrophic bacteria, 142A isolated from one of the lakes was chosen for this study as it could grow both at ambient and cold temperatures. The isolate was incubated at increasing concentrations of a reductant sulfide (63–188 ppm) at 5°C for 16 h and the viability estimated using Kogure's method[10,11]. Viability is expressed as percentage of total viable counts in the control observed after 16 h.

The TDCs in these oxygen supersaturated lakes were 20.45 × 10^8 cells l^{-1} whereas the percentage of direct viable aerobic and anaerobic counts were 7.6 and 88.6%, respectively. The retrievable anaerobic viable counts (RAnVCs) were 4 orders less than the TDC and 2 orders higher than the retrievable aerobic viable counts (RAeVCs). While the retrieval of total anaerobic counts was about 0.01%, that of TDLO and FB were 0.003 and 0.001 of TDC, respectively, i.e. ratio of distribution of AnB, TDLO, FB, SRB and AB in the total population was 100:30:10:10:1, respectively (Table 1).

The estimation of the total number of bacteria are in the same order 10^8 or 10^9, as described by Laybourn-Parry *et al.*[14] though they were 2 orders lower than that observed by Ramaiah[3]. Lower retrievability as compared to viability indicated that most of the cells were in viable but nonculturable state[15]. The high standard deviation encountered in the case of retrievable viable counts is indicative of the variability in twenty odd samples collected from different lakes (Table 1).

The solubility of oxygen in antarctic waters is high and is generally higher in the lakes than in sea water[16,17]. Ingole and Parulekar[5] reported that the dissolved oxygen in the fresh water lakes in Schirmacher oasis, East Antarctica, ranged from 10.4 to 13.8 mg l^{-1}. Dissolved oxygen in lake Priyadarshini alone, which is close to the Indian station, varied from 8.71 to 12.92 mg l^{-1} (ref. 18). Higher amounts of dissolved oxygen in these waters could be attributed to the higher solubility of the gas at lower temperatures.

Though oxygen is an effective electron acceptor, making energy conversion with high efficiency possible, it could be considered not so effective – or rather toxic

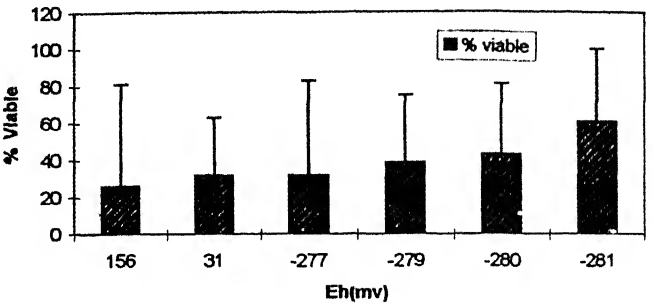

Figure 1. Bacterial viability at different Eh (n = 10 for oxidizing and 20 for reducing conditions).

when its concentration is high[19]. Even aerobic or facultative organisms live only in a balanced truce with oxygen[20]. Many strictly aerobic bacteria that form colonies from single cells on petridishes exposed to air can tolerate gas mixtures up to 40% oxygen by volume but fail to grow at 50%. Also 100% oxygen is usually considered to suppress growth. To many species of bacteria oxygen is inhibitory and causes a repellent response. Even obligate aerobes could be repelled by high concentrations of oxygen[21]. This is perhaps why the microorganisms have evolved a strategy to express increased viability only when the Eh is reduced. Perhaps they thrive better in anaerobic niches in highly aerobic water.

Experiments carried out on one of the isolates substantiate our field observations that viability is better expressed when conditions are reducing and temperature cold. The viability of cells incubated under aeration was only about 25% of the control when compared to 61% at 188 mv. Viability was maximum at 61% when the Eh ranged from –335 to –281 mv at 5°C (Figure 1). Only the Eh at the end of incubation is shown in the figure. Parallel experiments carried out at room temperature showed that solubility and therefore the availability of oxygen was lower. The expression of viability under anaerobic condition in these experiments was also lower (data not shown).

Thus, the field observations indicate that the potential expression of anaerobic growth by the bacterial population (viable and retrievable numbers) is much higher than the aerobic when the waters have generally high dissolved oxygen content. This paradoxical expression of increased anaerobic viability under oxygenated condition is more evident in the lacustrine environment than the marine. It is suggested that this phenomenon could be a strategy adopted by bacteria to express viability under reducing conditions when the concentration of dissolved oxygen in the surrounding waters is high/saturating. Further experiments are underway with microaerophilic, highly aerobic and strictly anaerobic isolates to understand the expression of viability in the highly oxidized environment.

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ACKNOWLEDGEMENT. We thank Ms S. M. Menezes, Goa University for technical assistance. A major part of the work was carried out during the 13th Indian Antarctic Expedition sponsored by Department of Ocean Development, GOI. NIO contribution no. 2654.

Received 19 December 1998; revised accepted 16 March 1999

BOOK REVIEWS

Fractals in Biology and Medicine, Volume II. G. A. Losa, D. Merlini, T. F. Nonnenmacher and E. R. Weibel (eds). Birkhäuser Verlag AG, PO Box 133, CH-4010, Basel, Switzerland. 369 pp. Price: DM 118/Fr 98.

Fractals were first introduced by Mandelbrot[1] while trying to find a solution to the seemingly simple problem of determining the length of the British coastline. He found that Euclidean concepts fail in these and other situations and that fractals were better suited for these purposes. Fractals typically (though not always) have a non-integer dimension and their fractal dimensions[2] are always greater than their topological dimensions. Further, fractals typically exhibit statistical self-similarity[2] over a range of length scales. Fractals can be used not only to describe objects but also to analyse time series data[3].

Fractals became very popular once their connection with chaos[4] was discovered in dissipative systems. It was found that attractors in chaotic dissipative systems are typically fractal in nature[4]. This led to many new methods of characterizing fractal dimensions[5]. Further, concepts like multifractals[5] became popular.

Fractals have now found applications in many areas of science and engineering other than chaotic dynamics[6–8]. Image compression techniques using fractals are quite common[6]. They have been used extensively in geophysics[9]. Recently, many applications have emerged in biology and medicine[10–14]. The book under review is an attempt to consolidate various new results in this exciting field of research. It contains papers presented at the Second International Symposium on Fractals in Biology and Medicine, held in Switzerland in 1996.

The first article in the book was presumably included to serve as an introduction to the field. It gives a brief overview of classical and stochastic dynamical systems from a mathematical viewpoint. Some remarks on applications of fractals in biology are thrown in (more as an afterthought). This article is too brief to be of much use to a reader new to the field. A more comprehensive introductory article would have made the book more self-contained. The rest of the articles can be classified into two broad types. Majority of them deal with the application of existing fractal techniques to classify and distinguish various types of cells. In particular, the use of fractal dimension to distinguish between malignant and benign cells is promising and could develop into an useful diagnostic tool to aid the pathologist. The other class of articles deals with application of dynamical systems and fractal theory to other biological subsystems. There is a sprinkling of articles on refinement of techniques, new fractal models, etc. There is even a highly speculative article proposing a binary theory of everything (taking a cue from string theorists) which claims to describe how complex structures of our Universe are generated!

I highlight below some of the interesting results from the first class of articles: In one article, using the spectral fractal dimension, the chromatin appearance (which is an important criterion used by pathologists in making a cancer diagnosis) is shown to be statistically self-affine. More importantly, the spectral fractal dimension is shown to differ significantly between benign and malignant cases leading to a correct diagnosis in 16 out of the 19 cases studied. In another paper, by measuring epithelial volume fraction of both fibrous mastopathy and mammarian cancer, significant differences are found using standard methods from dynamical systems theory. In a different article, fractal dimension using box counting method is shown to be effective in quantifying nuclear changes in MCF-7 human breast cancer cells when treated with steroid harmones. The same method is used in yet another article to analyse grey scale images of cells, but this time to distinguish between morphologically closely related malignant cells. Using local fractal dimension analysis, another paper characterizes tumour profile geometry quantitatively and further demonstrates that this approach gives a more accurate diagnosis as compared to human observers.

In the second class of articles, there is an interesting one which performs a dynamical analysis of the heart beat interval time series after cardiac transplantation. It is shown that the point correlation dimension drops to 1 after transplantation but increases with time to reach the normal value of around 5.4. A similar analysis is performed in a different article on the open and close time series of a large conductance Ca-activated K-channel. A non-integer point correlation dimension is obtained suggesting the presence of low-dimensional chaos. Another article demonstrates that arterial vasomotion patterns exhibit characteristics of nonlinear dynamical systems and show large-scale sensitivity to external perturbations. In another article, the fractal dimension is found to decrease immediately after a fracture (in sheep) and then increase to normal values as healing progresses.

To summarize, the book under review is an useful collection of articles on applications of fractals to biology and medicine. It, however, lacks a good introductory article which would have made the book more self-contained.

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Atlas of Carbonate Microfacies from the Reservoirs of Bombay Offshore Basin, India. K. Satyanarayana, R. R. Sharma, D . K. Dasgupta and K. K. Das. Regional Geology Laboratory, Exploration Business Group, Oil & Natural Gas Corporation Ltd., Mumbai, India. 1999. ISBN 81-7525-086-0. Price: US $290.

Oil exploration and exploitation are a technology- and money-intensive game that demand thorough in-depth analysis of all available geological and geophysical information. The game becomes more intricate while exploring a carbonate reservoir rock because of its mercurial susceptibility to diagenetic changes. These changes make it difficult to read correctly the depositional environment so essential in oil exploration and exploitation. Sedimentary environment controls the size, shape, composition, internal organization, position in a basin and bounding lithologies – be it either a carbonate or terrigenous sand bodies. Further, since diagenetic changes in carbonate rock occur at different stages of its evolution, the timing of such changes is important with respect to its structural changes and hydrocarbon migration.

Microfacies analysis of carbonate rock is essential in order to fully comprehend the composition, internal organization, post-depositional changes and evolution of porosity. The present book under review highlights these intricate microfacies changes in carbonate rocks of Bombay Offshore Basin in response to shift in depositional environment. The Oil & Natural Gas Corporation Limited (ONGC) has done a commendable service to the scientific community in publishing this atlas which will certainly help students and researchers engaged in the study of carbonate rock. Notwithstanding the fact that such an atlas is not new in the book market[1–2], what is new, however, is that (i) all illustrations are drawn from Indian sources, and (ii) ONGC has for the first time endeavoured to publish data that so long remained confined in their files. The price is, however, prohibitively high for anybody to procure a personal copy.

The atlas documents various microfacies and resultant porosity variations in carbonate reservoir rocks of various producing horizons of Bombay offshore oil basin. The illustrations are excellent. The authors have presented microfacies illustrations separately for various hydrocarbon bearing structures, e.g. Bombay High field, Deep continental shelf, Panna field, Bassein field, Mukta block, Neelam field, Heera field and Ratnagiri block. Illustrations of each field are preceded by a brief description of the salient geological features of the field, accompanied by generalized stratigraphic column and a map showing locations of wells drilled into the structure. It would have been instructive for the students if various log responses and structure contour and isopach maps had been presented for each field.

While providing a brief geological history of the Bombay Offshore Basin, the authors describe the basin as formed due to extensional tectonics. It is difficult to explain reverse fault in a pericratonic extensional basin (see p. 2). There are no evidences of it in the accompanying illustrations. It is also not clear how some homoclines and periclines are incorporated as 'basement structural elements' in the map.

The authors have thoughtfully included glossary of terms used in microfacies description and analysis. However, terminologies are not exhaustive and descriptions of those that are presented are very brief, casual and some are not correct. The term 'micrite' (acronym of microcrystalline calcite ooze) does not refer only to lithified carbonate mud (1–4 μm), but is used also as a synonym for modern carbonate mud. Further, in the glossary of 'micrite' and 'sparite' it should have been clearly mentioned that while the former can form a rock by itself with or without any association of allochems, it does not in case of sparite that forms cement in the pore spaces of allochems only. Sparite (if not neomorphic) does not exist independently. Authors have entered separate glossary for 'sparite' and 'sparry calcite'. What is the difference between the two? Why not simply define 'sparite' as a mosaic of crystals larger than those in micrite, formed either as cement or as neomorphic spar[3].

Again, since pellets cannot always be established as of fecal origin, it is better and safer to use a non-genetic term 'pelloid'. Keeping this in view, pelmicrite/pelsparite should be defined as a limestone composed of pelloids (allochem) in a matrix of micrite or sparry cement respectively.

The definitions of 'oomicrosparite', 'pelmicrudite' and 'pelsparrudite' in the glossary are misleading. Oomicrosparite means ooids set in a groundmass of homogeneous neomorphic spars, characterized by crystal sizes varying between 4 and 10 microns; it is not as given in the glossary. 'Oosparmicrite' may be a transitional type between oosparite and oomicrite where micrite from oomicrite may have been partially washed out with the resultant void spaces filled-in by calcite spar. In such cases, one has to be sure that calcite spars are not products of neomorphism. Again, since the size of the allochems is considered in determining the grain size name[4], it is not clear how pelloids can belong to rudite class (>1 mm).

The definition of 'packstone' has been defined casually as 'a limestone containing lime mud and particle supported'. It would have been better to define it as 'a grain-supported allochemical rock with carbonate mud matrix in the interstices'. The definition of 'pellet lime mud' is misleading and not clear. How could lime mud be 'shaped into sand-sized pellets'?

A mold, as defined in the glossary, cannot be natural impression but a pore formed by complete or selective removal by solution of a former individual constituent (allochem).

Coming to the illustrations in the atlas, none of them are numbered which is essential for reference and discussion purposes.

In legends of many of the illustrations, the word 'sparitization' has been interchangeably used both for neomorphic spars (microspars and pseudospars) and void-filling cements (see top and

bottom photographs on page 48 and top photographs on page 49). The term normally means neomorphism of micrite to spar.

There are shortcomings in the bibliography too. In the first place, unpublished ONGC reports should not have been cited since these are not normally available to the general readers other than ONGC scientists. Many of the citations do not contain page numbers. Peter Scholle's book was not published in 1998 but 1978.

It appears that the authors were in a hurry for its publication without giving much thought and care that publication of a book deserves. On the whole, the book is useful and instructive for students in their practical classes on carbonate petrography and the teachers too will find it handy for classroom instructions and illustrations.

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Annual Review of Microbiology 1998. Nicholas Ornston (ed.), Annual Reviews Inc., 4139, El Camino Way, P.O. Box 10139, Palo Alto, California. Volume 52. Price: Individuals $75; Institutions $150, 847 pp.

Modern biology is multidisciplinary by necessity. The *Annual Review of Microbiology* are an excellent resource series for readers interested not only in microbiology but also in genetics, molecular biology, cell biology and biochemistry. Since micro-organisms serve as excellent experimental organisms to understand basic biological phenomena, this review series occupies an important place. The current volume contains 21 wide-ranging reviews prefaced by Adelberg.

Living cells face varying environmental and nutritional conditions during their life cycle and for nutrient absorption, cell–cell interactions, etc. they form polarized cell structures. The yeast, *Saccharomyces cerevisiae* also exhibits polarized growth during starvation or mating and, therefore, serves as an excellent model system to understand these phenomena. It grows mainly as rounded cells but its pseudohyphal form allows yeast to adopt an invasive growth pathway in search of food under starvation conditions. The molecules involved in establishment of polarity and projections in response to pheromones, coordination of these events with cell cycle and cell fate determination by mother cell-specific expression of HO endonuclease mediated by specific localization of the *HO* gene repressor ASH1, are fascinating examples of differentiation mechanisms reviewed by Madden and Snyder.

Similarly, in the important area of aging research, yeast has shown the way. The mother cell buds to produce a smaller daughter cell but a given cell divides only for a finite number of times (25–30) before dying, exhibiting an age-related slowdown of cell cycle, onset of sterility and breakdown of nucleoli. Mutations that impart longevity to yeast are involved in cAMP metabolism, epigenetic silencing and genome stability. The common denominator in aging has been identified as accumulation of circular rDNA molecules. An understanding of the molecular basis may help in devising strategies to delay aging in humans. The advancements in this field are reviewed by Sinclair, Mills and Guarente.

Lantibiotics are the antimicrobial peptides made from modified building blocks like thioesters and thiazoles or unsaturated and stereoinverted amino acids and their post-translational modifications. The genes involved in their biosyntheses are organized in clusters. Sahl and Bierbaum describe how these novel antibiotics have dual functions of cell–cell signalling and immunity as well as antimicrobial activity. The last effect is exerted mainly through pore formation. Their properties can lend themselves to important applications.

The general view that bacteria exist solely as unicellular organisms needs revision as recent studies show that they do form highly differentiated multicellular structures through highly sophisticated signal transduction networks. Integration of intercellular signals leads to decisions about gene expression and cellular differentiation, in a manner similar to multicellular organisms. In three reviews the authors (Shapiro; Andrews; and Jacob, Cohen and Gutnick) discuss how the unicellular organisms can also adopt the multicellular state coupled with division of labour and harnessing of resources that cannot be effectively utilized by single cells, and for defense.

How cells adapt to environmental conditions is best exemplified by the glyoxylate bypass mechanism in enteric bacteria like *Escherichia coli*. This pathway is used to divert isocitrate from the TCA cycle when bacteria are grown in acetate rather than glucose to prevent the quantitative loss of acetate carbon as carbohydrate. It is governed by regulation of activity of isocitrate dehydrogenase by phosphorylation/ deposphorylation reactions, which helps to channel isocitrate through TCA cycle or glyoxylate bypass. This important mechanism is well reviewed by Cozzone.

A similar paradigm emerges from metabolic regulatory mechanisms of *B. subtilis*. Earlier considered a strict anaerobe, this soil organism is now known to adapt to anaerobic conditions, like water-logged soil, by turning on regulatory cascades (modifying a two-component signal transduction system) that allow the use of nitrate and nitrite as terminal electron acceptors. This is achieved by inducing the expression of fnr which, in turn, activates the genes involved in anaerobic metabolism. This interesting metabolic adaptation system is reviewed by Nakano and Zuber.

Most plants fix CO_2 by photosynthesis with the help of chlorophyll. However, the chemoautotrophic bacteria (mostly found under extreme environments and utilize sulphur, nitrogen, metals or carbon as electron donors) utilize the Calvin cycle for carbon fixation, in which one of the 13 main enzymes, ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBiSCO), is found in unique polygonal organelles called

carboxysomes. The regulation of genes involved in Calvin cycle and carboxysomes is well described by Shively, van Keulen and Meijer.

Micro-organisms also produce some novel enzymes that can be exploited for useful biotransformation reactions. Patel describes the novel approach of synthesis of paclitaxel, an anticancer drug (normally extracted from bark of Pacific yew trees at a very high cost) by biocatalysis with three novel enzymes obtained from the same plant species *Taxus*.

Modern world faces a serious problem of waste water treatment to remove several natural and man-made organochlorine compounds, that are causing severe and potentially irreversible ecological and environmental damage. Scientists are exploring ways of dealing with them. Lee, Odom and Buchanan review the normal measures involving microbial pathways of dechlorinating the toxic chlorinated aromatic compounds and converting them to nontoxic or metabolizable compounds. Other approaches to deal with the bottlenecks in microbial conversion of chloroaromatics involve genetic engineering of hybrid pathways, whereby genes for enzymes that degrade the toxic intermediates further, into the organism. These novel approaches and their mechanisms are reviewed by Reineke.

The specificity of transcription activation in prokaryotes is regulated by sigma (σ) factor that in turn controls the transcription of several housekeeping genes. In addition, several bacterial species undergo developmental changes like sporulation, flagellar secretion, stress response, etc. These developmental switches require new sets of genes to be expressed, which require specific σ factors. To achieve this, different bacterial species possess the anti-sigma factors, which abolish the function of the housekeeping σ factor and allow the stage-specific σ factors to express specific genes. The mechanisms of action of different anti-sigma factors from microorganisms like *E. coli*, *S. typhimurium*, *B. subtilis*, etc. is reviewed by Hughes and Mathee.

The unusual phenomenon of thymine less death (TLD) exhibited by bacteria, yeast and mammalian cells, involving cell death of thymine auxotrophs in response to thymine starvation, is reviewed by Ahmad, Kirk and Eisenstark. This exceptional phenotype is in contrast to biostatic effect of deprivation of other nutritional requirements and is caused mainly by loss of thymidylate synthase. It is accompanied by DNA damage in the form of single and double-strand breaks, which lead to cell death. The phenotype is exacerbated by mutations in recBCD pathway while recF repair counteracts it. This phenomenon is especially interesting since several anticancer drugs and antibiotics inhibit thymidylate metabolism and, therefore, understanding of TLD should help in devising better anticancer approaches.

Clostridium perfringens, the bacterium that causes human gas gangrene and food poisoning, produces several enterotoxins some of which are located on large extrachromosomal plasmids and transposons. Rood reviews the molecular biology of regulation of expression of enterotoxin by a two-component signal transduction system and the structure–function studies of enterotoxins.

Viruses exploit the cellular machinery for DNA replication, protein and RNA synthesis to propagate themselves. In addition, by a clever mechanism called virocrine transformation, viral gene products can activate the host cells' growth factor receptors (like erythropoietin) independently of their normal growth factor ligand, leading to host cell proliferation. Alternatively, their gene products may mimic the growth factor receptor thereby stimulating the host cell proliferation. This interesting phenomenon is reviewed by DiMaio, Lai and Klein.

The HIV retrovirus, the causative agent of AIDS, the most serious disease of epidemic proportions in the modern times, executes complex temporal programme of expression of late genes. This is mainly accomplished by the virus-encoded RNA-binding protein rev, which shuttles between nucleus and cytoplasm and plays an important role in transport of late viral mRNA from nucleus to cytoplasm. Because of its central role in regulation of HIV growth, rev also serves as a potential drug target. Mechanisms of regulation of rev and its role in RNA export are reviewed by Pollard and Malim.

The malarial parasite *T. brucei* has evolved intricate strategies for evading the defense mechanisms of the insect and human hosts, such that the parasite is able to extract its food from diverse hosts with the help of cell surface receptors (like transferrin receptors) and transporters (e.g. for LDL, HDL, etc.); however, these molecules are either not easily accessible to the host immune defence mechanisms or the organism can change its receptor and antigen receptors in a very small population, before the host can build up its defense mechanisms. These molecular strategies, as described by Borst and Fairlamb, should facilitate knowledge-based approaches for identifying new drugs and drug targets.

In conclusion, this volume covers several interesting areas like morphogenesis in prokaryotes, molecular biology of malarial parasite, viral gene regulatory mechanisms and excellent articles on morphogenesis control and aging control mechanisms in yeast. However some repetitions, like as many as three reviews on multicellularity in unicellular micro-organisms, could have been avoided.

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Annual Review of Earth and Planetary Sciences 1998. Raymond Jeanloz, Arden L. Albee and Kevin C. Burke (eds). Annual Reviews Inc., 4139 El Camino Way, P.O. Box 10139, Palo Alto, California. Volume 26. Price: US $75, 771 pp.

The present volume of the *Annual Review of Earth and Planetary Sciences* (*AREPS*) which contains nineteen articles, spans a broad array of research fields, from protoplanetary astronomy, marine geodesy, and metamorphic petrology to paleoecology, rock mechanics, and volcanology, the research fields which have made significant progress in the recent years.

Some of the greatest contributions to the geological sciences in the recent past came from geochronologists. They transformed the classical geology and freed it from its 'descriptive' mooring.

Numbers made a difference. For example, the Precambrian was a catch-all term for most of Earth's history, extending from about 600–4500 million years ago. Previously the Precambrians were subdivided into 'early' or 'late', a distinction that more often did not have any basis. Geochronological experiments provided ages of the rocks thereby facilitating mapping and correlation of the provinces. The prefatory article by George W. Wetherill ('Contemplation of things of the past'), presents a personalized view of the development of geochronological methods using mass spectroscopy in which the author was closely associated. The author narrates development of his career through 1950s and 60s, a period which saw blossoming of science in America. George Wetherill's contribution to geochronology has greatly helped geologists to understand the Precambrian geology. Another area where he has contributed much is the dynamics of stray bodies in the inner solar system, the meteorites and asteroids.

The present volume of AREPS contains a rather unusually long article (Steven B. Shirey and Richard J. Walker) on an exciting development in geochronology that is now taking place in Re–Os (rhenium–osmium) geochemistry. The development of high precision techniques provided the necessary boost to its analytical precision. The interest stems from the fact that Re and Os are highly siderophile elements, hence they strongly prefer metal or sulphide phases over silicate minerals. Consequently, Re–Os system can be employed to study cosmochemistry, chemical evolution of Earth's mantle and the origin of noble metal-bearing ore deposits, continental crust evolution, weathering, marine sediments and the chemical evolution of sea water. In another article, Kerry Gallagher, Roderick Brown and Christopher Johnson review the advances made in understanding the temperature dependence of fission track annealing and fission track length distribution (in apatite, zircon, and sphene). The past decade has also seen advances in fission track analysis which again is a geological dating tool. This technique has been applied to resolve a number of questions related to sedimentary provenance, thermal history of sedimentary basins, structural evolution of orogenic belts and continental denudation. The most common application is of course the dating of rocks. Over the past 10 years, studies have shown that fission track length is critical to the understanding of fission track ages and development of better annealing models. In its application to tectonism, better sampling strategies linked with structural mapping can provide better constraints on the evolution of fault zones.

Magellan data have been used in recent years to decipher the crustal and tectonic processes on Venus. The article on volcanism and tectonics on Venus by F. Nimmo and D. McKenzie synthesizes results from the recent work. This provides an opportunity to compare the planet's evolution with that of the Earth's. Interestingly, no features similar to spreading ridges were revealed from the synthetic aperature radar (SAR) images, indicating that plate tectonics is not active on Venus, except for localized rifting. Another interesting observation is that the faults associated with the rifts could be eight times more stronger than the faults on Earth. One possible reason for strong faults is the absence of water. Although there is no evidence of any overt crustal movements on Venus, mantle of the planet seems to be active with plume processes and mantle convections. The analyses of the SAR images indicate a global resurfacing event (basaltic upwelling) ending at 300–600 Ma. Ever since that catastrophic event, the planet has been heating up. The findings on Venus question the widely held assumptions of uniformitarianism. Size, composition, mantle temperature and lithosphere thickness of Venus are similar to Earth. However, a major controversy lingers on regarding the lithospheric thickness of Venus which has now become an active debate between Dan Mckenzie and Turcotte, two renowned geophysicists representing different schools of thought. Turcotte and others suggest a thick lithosphere (~ 200–400 km) and Mckenzie believes it to be a thinner crust (~ 100 km). Magellan gravity data apparently were not good enough to fully resolve this question. Finally, the question boils down to this: is Venus dying or will it come back to life sometime in future?

One example of catastrophism event on Earth may be the massive effusion of basaltic lavas during Cretaceous-Tertiary transition. An article, titled, 'The importance of pahoehoe' by S. Self, L. Keszthelyi and Th. Thordarson, reviews the advances made in the study of pahoehoe flows (with ropey surface texture, different from 'Aa' lava with blocky structure) which is the most common basaltic flow even in the continental flood basalt provinces. The recent volcanological studies have provided some understanding of the formation of these types of lava flows and specific processes involved in their emplacement via 'inflation', which was possible after several decades of observations. Inflated pahoehoe flows can attain great size because they are thermally very efficient. This is an important field of study as far as India is concerned. India has one of the largest basaltic lava (akin to pahoehoe) province which developed probably between 68 and 65 Ma with peak volume production lasting possibly < 1 Ma between 68 and 65 Ma. An important question raised in the article is the effect of large-scale eruption of pahoehoe lava flows on the global environment. There is now an incontrovertible evidence for a large 64.5 Ma bolide impact with lethal effects on global biota at a site called Chixulub, at the Cretaceous–Tertiary boundary which is somewhat coincident with continental flood basalt eruptions. The combined effect of these two phenomena could have dealt a body blow to global environment and biotic communities leading to mass extinctions.

An area which received a lot of attention in recent years is paleoclimatology. Fear of sudden climatic changes in the future was an added motivation in pursuing paleoclimatological researches. Proxy indicators including geophysical, geochemical, biological and sedimentological indexes have been used to characterize paleoclimatological changes. Summer and winter monsoon circulations are among the most dynamic interactions of the continent–ocean–atmosphere system on the globe. Extensive loess–soil sequences in north central China offer excellent sites to study the spatial and temporal changes of monsoon during the Quaternary. Chinese scientists have made significant contributions to this study. A major finding is that the temporal and spatial

changes in monsoon in the Quaternary can be linked to global ice-volume variations. In the article, 'Chinese loess and the paleomonsoon', Tungsheng Liu and Zhongli Ding make succinct review of the researches carried out in this field in which most of the original papers are published in Chinese. We have a stake in these studies because India's development largely depends on a steady monsoon. We should be working with the Chinese scientists on these aspects.

Recent years have seen a much more insightful understanding of the processes of faulting and the influence of the internal structure of the fault on earthquake processes. Chris Marone's paper 'Laboratory derived friction laws and their application to seismic faulting' presents models that relate after-slip to the structure of the fault zone, using examples from the San Andreas fault. Although the models explain the absence of slip within the main rupture, the role of gouge in limiting the after-slip remains an open question. A spectrum of behaviours ranging from aseismic creep to accelerating slip or slow precursive slip prior to fully dynamic instability is governed by friction laws. However, as the author himself points out, the field observations and models need to be tested by specific laboratory studies and development of theoretical models.

Richard G. Gordon's article on 'the plate tectonic approximation' deals with basic assumptions that underlie the theory of plate tectonics. The validity of the two central assumptions on the rigidity of the plates and the narrowness of the boundaries forms the theme of this paper. Apparently, both these assumptions are contradicted by many observations, both in continents and oceans. In his reconstruction based on the strain rates and relative velocity of plates, diffuse plate boundary zones cover 15% of earth's surface. While some important issues on rigidity of the plates and misfits of plate reconstruction remain unclear, Gordon makes no effort to examine the global seismicity and stress patterns, considered as clear manifestation of plate deformation.

The metamorphic terranes of south India offer excellent type areas for studies of charnockite formation and its association with CO_2 infiltration. Researches into these aspects both by Indian and non-Indian scientists provided a better insight into the nature of transportation during metamorphism. John M. Ferry and Martha L. Gerdes base their arguments on those findings and other work and highlight the stable isotopic, mineralogical, and chemical alterations during metamorphism as evidence for reactive fluid flow. However, the fact remains that as far as granulites are concerned, the role of reactive fluid flow continues to be controversial because transportation can be both by fluid flow (advection) and by diffusion within the fluid or the solid state. Isotopic, mineralogical and chemical data from granulite terrane of south India suggest discrete development of granulite along fractures indicating infiltration of CO_2-rich fluid. In contrast, studies in the Adirondack area record fluid-absent conditions in the granulite development. Future studies will try to resolve this by improving the database on both chemistry and mechanics of fluid flow.

There has been a significant growth in the application of advanced technologies in earth sciences. The advent of modern satellites has revolutionized the methods of data gathering and management. Two exhaustive articles in AREPS deal with use of satellite in deciphering sea floor tectonic fabric and oceanic circulation. Besides, there are papers on the study of presolar grains from meteorites which in fact is star dust (how poetic this word is!) that are ejected from supernova prior to the formation of solar system. Laboratory studies of these grains provide information on stellar evolution. Another fascinating article deals with early history of insect–plant association. In short, the 1998 AREPS volume covers a wide range of topics in earth and planetary sciences, most of them on emerging research areas. These papers not only provide an introduction to the respective research fields, but also serve as a gateway to the current literature. We have no hesitation in recommending this book to the students as well as the professionals.

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Illustrated Text Book on Sericulture. Translated from Japanese: Zukai Sangyo Dokuhon. Japan Sericulture and Agriculture Cooperative Association Confideration, Tokyo. 1967, 159 pp. Translated by Alamelu Gopal, Technical Editor, D. Mahadevappa. 1998. Mohan Primlani for Oxford & IBH Publishing Co. Pvt. Ltd, 66, Janpath, New Delhi 110 001. Price: Rs 275.

As stated by the Board of Editors (May 1967) in the Preface of the book, this is a textbook of sericulture technique(s) illustrated with detailed photographs, figures and tables. The attempt is really refreshing as the reader gets easily familiarized with the subject matter. What is, however, disappointing is that the matter presented in 1967 is being translated into English only in 1998, almost 31 years later; although the editors say that the latest information is given, the 1967 edition does not quote 'revised'. The entire subject of the textbook is exclusively applicable to Japanese sericulture only.

The first chapter, mainly devoted to mulberry cultivation (65 pages), is justifiable because the culture of mulberry (moriculture) forms the basic foundation of sericulture. Mulberry leaves form the sole food material of commercial silk producing silkworm *Bombyx mori.* L. The success of sericulture industry is mostly dependent on good quality mulberry leaves. The relationship of leaf quantity and yield to number of branches and unit branch length is well discussed with tabular representations. Detailed information about mulberry varieties is compiled. Planning of mulberry fields and their establishment with respect to young age and late age silkworm rearing are neatly presented. Easy and simple methods to determine the planting distance are shown. An account of various methods of training and harvesting of mulberry for silkworms of different maturity (young and adult) is beautifully illustrated. There are photographs depicting mechanization of mulberry field management.

The diseases and pests of mulberry and their management are covered in detail. This is an important aspect of mulberry cultivation. But many shortcomings are noticed. To cite a few: the photographs do not clearly show the

disease symptoms as they are in 'black and white'. The author could have used glossy colour prints for clarity. The causal agents of diseases are not mentioned. The labelling pattern is not uniform. For example, if (A), (B), (C), etc. are used in the text, in figures legend (a), (b), (c), etc. are used. Although minor, such mistakes can confuse the reader. There are also a few serious technical mistakes. Under powdery mildew disease, the ascocarp is a cleistothecium and not perithecium (p. 50). The white root-rot pathogen is an ascomycetous fungus *Rosellinia necatrix*. The legend for figures is wrongly written as Bacidiospore (p. 52). These are formed only in Basidiomycetous fungi and in ascomycetous fungi, ascospores are produced. Also, the term bacidiospore is used without any discretion. For example, the macro- and microconidia are wrongly labelled as Conidiospores and Bacidiospores, respectively (p. 53).

The life cycle of different insect pests of mulberry is well covered. However, one is not sure whether BHC is still not banned in Japan as it is advocated under chemical method of controlling mulberry small weevils as well as uzi fly menace. The safety period to be followed after chemical sprays is not mentioned.

The second chapter is on silkworm rearing (pp. 66–112). The life cycle of silkworm, its morphology, physiology and embryology is illustrated in a simple manner. Silkworm egg production, artificial hatching methods, incubation schedules and rearing plans are all appropriately described. A detailed account of different types of disinfection methods is given. An easy method of selecting and cutting the mulberry for young silkworms during different seasons (summer and autumn) is nicely illustrated (p. 80). Rearing methodology for young silkworms and co-operative rearing – merits and demerits, requirements are briefly explained. Useful information is provided for outdoor rearing of grown-up silkworms. Mechanization of rearing is also covered.

Under diseases of silkworm, except 'Aspergillosis', the causative agent(s) is not mentioned here also. However, the information provided under fungus (Muscardine) disease of silkworm and the relationship with other insects in the field is interesting and useful. The legend (A, B, C, D) used for photographs is not indicated in the text. One may find it difficult to identify them correctly unless one is an entomologist. The electron microscope photographs of virus particles although good do not mention the magnification.

The third chapter is on management (pp. 113–128). Several features of sericulture farms such as profitability of sericulture compared to other agricultural crops are worked out in a simple way. Useful tips are provided regarding management improvement with minimum labour. The chapter also contains useful information on the operational efficiency of sericulture in terms of mulberry cultivation and silkworm rearing. Productivity of sericulture from 1960 to 1965 in 10 prefectures is calculated. Details of expenditure on cocoon production are illustrated by taking the average of 974 farms all over Japan.

The fourth and the last chapter gives general information (pp. 129–145). A map of world sericulture indicates the cocoon and raw silk quantities produced in various countries. A good compilation of sericulture-related authorities, agencies and organizations is presented (pp. 131–132). While dealing with the sericulture of Japan, 'present status' referring to data as old as 32 years (1966) should have been updated. The entire technological aspects of 'silk' are drastically condensed and given in a nutshell. Sale of cocoons and methods of fixing cocoon price as followed in Japan are outlined briefly. The different steps involved from cocoon to production of raw silk fibre are picturized and oversimplified. This particular aspect needs details from the point of view of a student, particularly because this book is titled as text. It is nowhere clear whether the contents of this textbook pertain to the syllabus of any course/study in Japan.

Despite several short-comings, simplicity of presentation makes it a good reading material worth possessing. It is particularly useful for beginners as it is beautifully illustrated.

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Magnetic properties of solids: Krishnan's contribution

C. K. Majumdar

K. S. Krishnan and his students developed precise experimental methods to measure magnetic anisotropy of crystals. The work established the usefulness of magnetic methods as a valuable supplement to X-ray methods for determining crystal structure. This led to the organization of the Department of Magnetism in the Indian Association for the Cultivation of Science. Some remarks about the subsequent developments in the field of magnetism will be made to put the work in modern perspective.

K. S. Krishnan[1] came to Calcutta in 1920. The capital of India in the British empire had been moved to Delhi in 1912. The University College of Science and Technology at Rajabazar in Calcutta was founded in 1914 by the efforts of Asutosh Mookerjee and with munificent contributions from Taraknath Palit and Rashbehary Ghosh. When the aura of political power faded, the stars in Calcutta's science began to shine more brilliantly. C. V. Raman was Palit Professor of Physics and had his research group in the Indian Association for the Cultivation of Science (IACS) at 210 Bowbazar Street. For Krishnan, the star stood over this address. He joined Raman's group, and from 1923 to 1928 he took part in spectroscopic researches, which included the discovery of the Raman Effect.

The other professor at the Calcutta University Physics Department was D. M. Bose, who was working on magnetic materials at that time. Bose's researches led to the 'spin-only' formula for the magnetic moment for the 3-d atoms in salts; this won international recognition[2,3]. The next important contribution in the field of magnetism came from K. S. Krishnan and his students first at the Dacca University (now spelt Dhaka in Bangladesh) and later at IACS. They carried out measurements of magnetic anisotropy in crystals by the elegant oscillation method[4] and the critical couple method[5].

Magnetism in crystals

The magnetic energy of a crystalline substance can be written as

$$E = -\frac{1}{2}\int [k_{11}H_x^2 + k_{22}H_y^2 + k_{33}H_z^2 + 2k_{12}H_xH_y + 2k_{23}H_yH_z + 2k_{31}H_zH_x]\,dv, \quad (1)$$

where k_{11}, k_{22}, etc. represent the susceptibilities of the crystal referred to the coordinates x, y, z, and the integral is taken over the whole volume of the crystal, whose shape is not yet specified. When the applied field is in the x-direction, $H_y = 0$, $H_z = 0$ and the energy is

$$E = -\frac{1}{2}\int k_H H^2 dv, \quad (2)$$

where k_H is the susceptibility of the crystal in the x-direction and depends on the direction x of H. The magnetic energy cannot depend on the choice of axes. Hence

$$k_{11}H_x^2 + k_{22}H_y^2 + \cdots + 2k_{12}H_xH_y + \cdots = k_H H^2. \quad (3)$$

If (l_1, m_1, n_1) are the direction cosines of H relative to the coordinates (x, y, z) we get

$$k_{11}l_1^2 + k_{22}m_1^2 + \cdots + 2k_{12}l_1m_1 + \cdots = k_H. \quad (4)$$

Now we may refer the magnetic ellipsoid to its principal axes which are defined by $k_{12} = k_{23} = k_{31} = 0$, $k_{11} = k_1$, $k_{22} = k_2$, $k_{33} = k_3$. k_1, k_2, k_3 are termed the principal magnetic susceptibilities. Then if H is in the direction (l, m, n) we get

$$k_1l^2 + k_2m^2 + k_3n^2 = k_H. \quad (5)$$

Corresponding to the volume susceptibilities k_1, k_2, k_3 we have mass susceptibilities χ_1, χ_2, χ_3, respectively. Measurement with powdered samples gives us $\frac{1}{3}(k_1 + k_2 + k_3)$ or $\frac{1}{3}(\chi_1 + \chi_2 + \chi_3)$.

On exciting an inhomogeneous magnetic field the force on a small crystal of volume v is

$$k_H v H(dH/dx). \quad (6)$$

A paramagnetic substance tends to move to a higher field. By measuring the force the susceptibility can be measured.

It often happens that in the case of a monoclinic crystal, the direction of only one principal magnetic axis k_3 is known. If the crystal is mounted with the k_3 axis parallel to the z-axis and perpendicular to H, then $n = 0$, and

$$k_1l^2 + k_2m^2 = k_H$$

or,

$$k_1\cos^2\phi + k_2\sin^2\phi = k_H, \quad (7)$$

where ϕ is the angle between the k_1 axis and H. Hence, a couple (equal to the rate of change of magnetic energy with angle of displacement, $dE/d\phi$) will act on the crystal:

$$\frac{dE}{d\phi} = -\frac{1}{2}\frac{d}{d\phi}\left(k_H H^2 v\right)$$

$$= \frac{1}{2}(k_1 - k_2)vH^2\sin 2\phi. \quad (8)$$

When a circular disc of the crystal is cut with the plane of the disc perpendicular

to the known magnetic axis and suspended with the plane horizontal and parallel to a uniform field H, the disc will be acted upon by a couple given by eq. (8), which is maximum when $\phi = 45°$.

Experimental methods

Oscillation experiments

Suppose that a crystal is suspended by a torsion fibre parallel to the k_3 axis and oscillates with a period T_0. If now a uniform field H is applied parallel to the k_1 axis, then from eq. (8) on rotating the crystal through an angle $\delta\phi$ about the axis of the fibre, a restoring couple $\{C + (k_1 - k_2)H^2v\}\delta\phi$ acts on the crystal instead of the couple $C\delta\phi$ in the absence of the field, where C is the torsion constant of the fibre. A new period of oscillation T_1 is observed. Then

$$CT_0^2 = \{C + (k_1 - k_2)H^2v\}T_1^2, \qquad (9)$$

so

$$k_1 - k_2 = ((T_0^2 - T_1^2)/T_1^2)(C/H^2)(1/v), \qquad (10)$$

or

$$\chi_1 - \chi_2 = ((T_0^2 - T_1^2)/T_1^2)(C/H^2)(1/m), \qquad (11)$$

where m is the mass of the crystal. If χ is gram molecular susceptibility, the right hand side of eq. (11) is multiplied by the molecular weight M.

Krishnan and his students attached each crystal to the lower end of a short piece of glass suspended by a vertical quartz fibre from a torsion head and found the periods of oscillation. Complications from the shape of the specimens were attended to. Special attention was required to ensure the uniformity of the magnetic field (~5 kG).

The value of the largest principal susceptibility was found by the Rabi immersion method[6]. A quartz fibre was used; the solution was weak manganese chloride.

Critical couple method

The crystal is suspended in a uniform horizontal magnetic field at the end of a calibrated long, thin quartz fibre, the upper end of which is fixed to the axis of a graduated torsion head. The crystal is allowed to take up its equilibrium orientation in the field under zero torsion of the fibre. If the torsion head is now slowly rotated through an angle α, the crystal will rotate in the same direction but through a smaller angle $(\alpha - \phi)$. The couple acting on the crystal tending to restore it to its original orientation would be equal to $(m/2M)\Delta\chi H^2\sin 2\phi$, according to eq. (6), where $\Delta\chi$ is the difference between the maximum and minimum gm molar susceptibilities of the crystal in the horizontal plane. This couple is balanced by that due to the torsion fibre, viz. $C(\alpha - \phi)$. As the torsion head is rotated further, there comes a stage when ϕ just reaches the value $\pi/4$ (the corresponding value of α being α_c, say), and the couple due to the magnetic field reaches its maximum value. Equating the opposing couples, we get

$$C(\alpha_c - \pi/4) = \frac{m}{2M}\Delta\chi H^2. \qquad (12)$$

With the slightest further rotation of the torsion head the crystal will naturally yield and turn around. On this property is based an accurate measurement of α_c which by eq. (12) enables us to determine $\Delta\chi$. In practice $2\alpha_c$ is measured directly by finding the two critical positions of the torsion head, obtained by clockwise and anticlockwise rotations of the torsion head from its initial position.

We have only described the main experimental methods. The papers of Krishnan and coworkers contain lots of experimental tricks to measure weak anisotropies. They contain enormous amount of data.

These papers established Krishnan's idea that the magnetic anisotropy of a diamagnetic or paramagnetic crystal could be correlated with the anisotropy of the individual molecules and their relative orientations. In favourable cases the precise orientation of molecules in the unit cell could be determined from magneto-crystalline measurements. The work of Krishnan and his students, and of Kathleen Lonsdale established the fruitfulness of magnetic methods as a valuable supplement to the methods of X-ray analysis for determining the architecture of crystals.

Later work on iron group and rare earth salts correlated with theoretical work of Van Vleck and Penney and Schlapp on crystal fields in solids.

Another important work by N. Ganguli and Krishnan established that electrons in graphite form a two-dimensional electron gas obeying Fermi–Dirac statistics[7].

Subsequent developments

We note that Krishnan's measurements were started in a small university department and were done with an apparatus not too expensive. Today a university department can afford the latest version of the apparatus at IACS (ref. 8) which operated between liquid air temperature and a moderately high temperature. But investigations in magnetism subsequently developed in directions well beyond the capacity of university departments[9].

Firstly, the magnetic phenomena require high fields and liquid helium or even lower – millikelvin – temperatures. Perfect diamagnetism is found in superconductors. Superconducting magnets, based on type-II superconductors, can produce very high, steady fields. These are beyond a university department. Only with the discovery of high temperature (above liquid nitrogen temperature) superconductivity, research in such a subject can be done in university departments. Some departments in universities and IITs have procured ..ra tion magnetometers and carried on research in magnetic materials.

Secondly, magnetic structure determination took a new turn with the introduction of neutron methods. Neutron diffraction and neutron scattering methods can only be done in the vicinity of reactors. Universities or even research institutes could not carry out such experiments. The record of research at Trombay reactors at BARC, Mumbai, has been good. Recently with the establishment of Inter-university Centres for Department of Atomic Energy Facilities (IUC–DAEF) university workers can hope to do neutron-based work.

Thirdly, research on nuclear moments, much smaller than atomic moments, started with atomic and molecular beams and later gave rise to nuclear magnetic resonance (NMR)

methods and other related resonance methods. Only research institutes could afford such facilities in India; in particular, the uniformity of magnetic field in NMR studies required high technology. Still several research groups have procured such facilities and done good work. Only the Mössbauer experiments could be done in many laboratories in universities, provided the source could be obtained from reactors or accelerators.

The style of doing research in magnetism has changed, but the standard set by Krishnan and his students has not been surpassed or equalled.

Some information about Krishnan's students in magnetism may not be out of place. B. C. Guha joined (West) Bengal Educational Service and was a part-time teacher in Physics in Calcutta University when I was a student in the M Sc class (1958–60); he used to lecture on magnetism. A. Mookherjee was (probably) the teacher in St. John's College, Agra (1959) who showed us around his laboratory in magnetism when we, the students of physics from Calcutta University, visited Agra on an educational tour (1959); later he taught at Burdwan (now spelt Bardhawan) University. Santilal Banerjee also joined (West) Bengal Educational Service and taught in Presidency College, Calcutta, in 1940; his name appeared in the college records[10]. A. Bose stayed in IACS, continued magnetic measurements at low temperature and later built up the Indian Cryogenic Council; he was a Fellow of the Indian National Science Academy, Delhi. S. C. Ganguly taught at Bangabasi College, Calcutta, and later at Jadavpur University. A. C. Guha taught in Berhampur College at Berhampur, West Bengal, and is still alive.

1. Krishnan, K. S., *Collected Works of K. S. Krishnan*, National Physical Laboratory, New Delhi, 1988.
   Two amusing slips in the excellent biographical sketches, one by K. R. Ramanathan and another by K. Lonsdale and H. J. Bhabha, should be noted: S. N. Bose never wrote a thesis for a formal doctoral degree; in Krishnan's days football matches were played not in the Eden Gardens, but in the 'Maidan' or 'Garer Math' (playgrounds outside the Fort).
2. Van Vleck, J. H., *The Theory of Electric and Magnetic Susceptibilities*, Clarendon Press, Oxford, 1932.
3. Bates, L. F., *Modern Magnetism* Cambridge, 1951.
4. Ref. 1, p. 308.
5. Ref. 1, p. 402, also p. 439.
6. Ref. 1, p. 346; also ref. 3.
7. Ref. 1, p. 509.
8. Ref. 1, p. 580 shows a figure of this apparatus. The earlier papers did not give any picture of the apparatus.
9. See e.g. Majumdar, C. K., in *Current Trends in Magnetism* (eds Satya Murthy, N. S. and Madhav Rao, L.), Indian Physics Association, 1980.
10. Presidency College, Calcutta, Centenary Volume 1955 (West Bengal Govt. Press, Alipore, 1955), p. 54 [Teachers in Natural Science – Physics].

ACKNOWLEDGMENTS. I must thank Prof. Bejoy Sankar Basak, former Principal of Presidency College, Calcutta, for information about K. S. Krishnan's students at IACS. Part of this paper was presented at the Krishnan Conference at the Allahabad University, December 1998. I should also thank S. N. Bose National Centre for Basic Sciences for providing facilities to complete this manuscript.

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Erratum

Looking for C. V. Raman? Hunt for the likes of Asutosh Mookerjee first

S. K. Bhattacharjee

[*Curr. Sci.*, 1999, **76**, 862]

2nd column, 2nd para, 11th line:

"only in gaseous phase molecules".

should read:

"only in quartz crystal."

Edited and published by Prof. P. Balaram and Prof. S. Ramaseshan, Current Science Association, Bangalore 560 080.
Typeset by WINTECS TYPESETTERS (Ph: 3327311), Bangalore 560 021, Printed at Printek Printers, Bangalore (Ph: 3357763).

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CSIR, the premier agency established by the Govt of India to undertake scientific and industrial research in the country, is seeking to appoint in its coveted top level S&T Management Cadre a Director for its Central Scientific Instruments Organisation (CSIO), Chandigarh. CSIO undertakes research, design and development of scientific and industrial instruments and instrument systems; provides technical assistance and services such as fabrication, repair, maintenance, testing, calibration, analysis and performance evaluation of instruments and develops human resources for instruments and related industries.

CSIO has three service and maintenance centres at New Delhi, Jaipur and Chennai and runs an Indo-Swiss training centre at Chandigarh. CSIO has a total staff strength of around 800 including 115 scientists. The annual budget is around Rs 15 crore, of which nearly 30% is derived from contracted R&D work, services and consultancy.

The Director is the Chief Executive of the Institute and is responsible among others for realizing CSIO's mission of helping the country move towards an instrumentation efficient nation.

Qualifications for the post: M.Tech/M.E. with 14 years of relevant experience or Ph.D (Science) with 13 years of relevant experience or Ph.D (Engg) with 12 years of relevant experience.

The candidate must be a creative, innovative and well-established scientist/technologist of distinction in the areas of activities of CSIR and should preferably be around 50 years of age. The applicant should possess leadership qualities covering *inter-alia*, a demonstrated ability to create an environment conducive to nurturing of high class R&D talent, a proven record of inter-personal skills and an ability to communicate effectively.

Pay, allowances and facilities

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The appointment to the post will be on contract for a period of six years or up to superannuation (at the age of 60 years) whichever is earlier. The contract can be extended in exceptional cases.

Interested candidates may send their complete biodata by 30 July 1999 to the Director-General, CSIR, Rafi Marg, New Delhi 110 001 (Fax: 3710618; E-mail: dgcsir@csirhq.ren.nic.in).

INDIAN SCIENCE CONGRESS ASSOCIATION

YOUNG SCIENTISTS AWARD PROGRAMME

To encourage young scientists, the Indian Science Congress Association under its above programme introduced a number of awards in January 1981. Each award carries a cash amount of Rs 5,000/- and a certificate of merit.

1. Only members of the Association are eligible for consideration for the award. The upper age limit of the candidates for the award is 32 years (as on 31 December 1999 – not *1988* as published earlier [*Curr. Sci.*, 1999, **76**, 1400]).

2. Two copies of biodata, including full name and address along with the date of birth (duly supported by attested copy of the certificate), membership status and number, research experience (in case of joint authorship, the candidate has to be acknowledged by the other author(s) in terms of a certificate as having made the major contribution) and certification that the work has been carried out in India and has not been submitted for any award, should be appended to the complete paper.

3. Three copies of full papers along with their abstracts in triplicate (not exceeding 100 words) should reach the office of the General Secretary (Head Quarters), Indian Science Congress Association, 14, Dr Biresh Guha Street, Calcutta 700 017, not later than 20 September 1999. At the top of each copy of the paper and its abstract, the name of the Section where the paper is to be presented, should be indicated.

4. A young scientist could present only one paper in only one section (and not a second paper with the same or any other context in any other section) for the year under consideration.

5. Full papers will be assessed for their content and at most 6 young scientists in each section will be invited to make an oral presentation of their papers during the Science Congress Session. They will be provided with admissible travelling and daily allowances by the ISCA (maximum of first class train fare by convenient shortest route to and from residence/Institute to venue and DA as per ISCA rules).

6. The final selection for the awards will be done by a duly constituted committee and will be announced on the last day of the congress.

Central Glass & Ceramic Research Institute

196, Raja S. C. Mullick Road, Calcutta 700 032, India

Notification No. 5/GC-(R&C)/BRNS/99 Date: 1.06.99

Applications are invited from bonafide Indian citizens for the post of a Senior Research Fellow and a Project Assistant for working in a project entitled 'Development of high damage resistant sol-gel coatings for high power laser' tenable at CGCRI, Calcutta. The tenure of posts is 3 years which will be terminated at the end of the 3rd year.

Post	(i) Senior Research Fellow – 1 (ii) Project Assistant – 1
Stipend	(i) Rs 5600/- (for 1st and 2nd year) plus HRA Rs 6000/- (for 3rd year) plus HRA (ii) Rs 5000/- (consolidated for 3 years)
Qualifications	For post No. (1) M. Sc. in Chemistry (specialization preferably in Inorganic or Physical Chemistry)/M.Sc. in Physics (specialization preferably in Solid State Physics) or B.E./B.Tech. in Ceramic Technology/Chemical Engineering with two years experience. OR M.E./M.Tech. in Ceramic Technology/Chemical Engineering/Optics and Optoelectronics.
For post No. (2)	M.Sc. in Chemistry (specialization preferably in Inorganic or Physical Chemistry)/M.Sc. in Physics (specialization preferably in Solid State Physics) or B.E./B.Tech. in Ceramic Technology/Chemical Engineering.
Age	For post No. (1): Upper age limit is 32 years as on 01.06.1999 For post No. (2): Upper age limit is 28 years as on 01.06.1999
Brief job description	Systematic study on thin film deposition on BK7 and phosphate laster glasses for high damage resistant antireflection (AR) and high reflection (HR) effects for high power laser applications in Department of Atomic Energy, Govt. of India.
Other terms and conditions :	BRNS/CSIR terms and conditions will normally apply.

Applications on plain paper giving complete bio-data (i.e. name, date of birth, father's name, present and permanent address, educational qualifications, experience, etc.) should reach the Controller of Administration, Central Glass and Ceramic Research Institute, 196, Raja S.C. Mullick Road, Calcutta 700 032 by 19 July 1999.

ASTRA RESEARCH CENTRE INDIA AND CAMBRIDGE HEALTHTECH INSTITUTE USA

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There is a major paradigm shift in drug discovery research due to advances in Life Sciences, Chemistry and Information Sciences. Enabling technologies for cloning and expression of target genes, Development and miniaturization of biochemical assays, High throughput screening, Combinatorial chemistry, Parallel organic synthesis, Robotics and informatics are providing new opportunities for research towards the discovery of novel therapeutics. Distinguished scientists from industry and academia will present latest developments in these topics. The symposium is designed to benefit senior scientists, project leaders, decision makers and students of pharmaceutical sciences and Life Sciences.

Registrations are open to researchers from pharmaceutical R&D organisations, University scientists and students. Early registration is advised.
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Write to: Director, Astra Research Centre India, P.O. Box 359, Malleswaram, Bangalore 560 003
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DEPARTMENT OF CHEMICAL TECHNOLOGY UNIVERSITY OF MUMBAI MATUNGA, MUMBAI 400 019

Applications are invited from Indian nationals for the position of a Junior Research Fellow under a scheme entitled 'Studies on processing and utilization of gamma irradiated oilseeds' sponsored by the Department of Atomic Energy, Government of India.

No. of positions	1 (one)
Prescribed qualifications	B.Sc. (Tech.) degree in Oils Technology or M.Sc. degree in Organic Chemistry
Remuneration	Rs 5,000/- p.m. and HRA as per rule.

NET/Gate qualified candidates will be preferred. Candidates awaiting the result of qualifying examinations are also eligible to apply. Selected candidates may register for higher degree subject to the approval of post graduate selection committee. Please send your application to the Director (Attn: Dr D. N. Bhowmick), University Department of Chemical Technology, Matunga, Mumbai 400 019 latest by 19 July 1999.

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Advt. No. 11/99 14 June 1999

Applications are invited for the post of Faculty Fellow (equivalent to Assistant Professor in IIT/IISc) in the Evolutionary and Organismal Biology Unit. Applicants must have a Ph.D. degree with good post-doctoral experience and a sound record of publications. The current focus of the Unit is in the areas of Chronobiology, Behavioural Ecology, Evolutionary Genetics and Population Dynamics, Microbial Ecology and Biodiversity.

The Centre is looking for candidates with strong motivation and with a proven record of independent research and teaching.

The post carries a basic pay of Rs 12,000 in the grade 12,000–420–18,300. (The total minimum emoluments will be approximately Rs 17,880 per month).

Applications may be sent to: The Coordinator, Jawaharlal Nehru Centre at the address given above, on plain paper along with a detailed *curriculum vitae*, copies of the best five publications, a tentative research proposal and names of at least three referees.

Last date for receipt of applications: 30 July 1999.

JAWAHARLAL NEHRU UNIVERSITY NEW DELHI

Applications are invited for a Junior Research Fellow (JRF) to be appointed in the research project entitled 'Mechanism of Action of Photodynamic Herbicides' sponsored by the Department of Atomic Energy. Applicants should have obtained first class M.Sc. degree in Life Sciences/Botany/Biochemistry/Allied sciences. Applications should reach Dr B. C. Tripathy, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067 within 15 days of publication of the advertisement.

INFORMATION FOR CONTRIBUTORS

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All manuscripts should be addressed to the Editor, *Current Science*, P. B. No. 8001, C. V. Raman Avenue, Bangalore 560 080. Submission of an article will be held to imply that it has not been previously published and is not under consideration for publication elsewhere; and further, that if accepted, it will not be published elsewhere. *Three copies of contributions of all categories* are required, with a letter of transmittal giving (i) names and complete addresses of the authors and (ii) title of the contribution and the category in which it is submitted (see below).

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Manuscripts should be typed double-spaced on one side of white bond paper (21 × 28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The **title** should be brief, specific and amenable to indexing. Not more than five **keywords** should be indicated separately; these should be chosen carefully and must not be phrases of several words. **Summary** and **abstract** should not have more than 100 words and should convey the main point of the paper, outline the results and conclusions, and explain the significance of the results.

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319–322.

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